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低鱼粉饲料中添加牛磺酸对青鱼幼鱼生长、肠道修复及抗急性拥挤胁迫的影响

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摘要: 为研究低鱼粉饲料中添加牛磺酸对青鱼幼鱼[初始质量(5.90 ± 0.03)g]生长、肠道修复及抗急性拥挤胁迫的影响, 实验以青鱼正常鱼粉组(20%)为对照组, 10%鱼粉组为负对照组(I_0 组), 在低鱼粉饲料中分别添加0.05%($I_{0.05}$ 组)、0.1%($I_{0.1}$ 组)、0.2%($I_{0.2}$ 组)、0.4%($I_{0.4}$ 组)牛磺酸, 配制6种等氮等脂饲料, 饲养8周。饲养实验结束后, 参考生长结果, 选择对照组、 I_0 组、 $I_{0.1}$ 组、 $I_{0.4}$ 组进行急性拥挤胁迫实验。结果显示: ①与对照组相比, I_0 组增重率显著下降, 随着低鱼粉饲料中牛磺酸水平的升高, 青鱼幼鱼增重率呈先上升后下降趋势, 当牛磺酸添加量为0.1%时与对照组差异不显著; ②相对于对照组, I_0 组的绒毛高度降低、隐窝深度增加, 杯状细胞数降低, 添加牛磺酸使绒毛高度升高、隐窝深度降低, 杯状细胞数和淋巴细胞数增多; ③急性拥挤胁迫使青鱼幼鱼血清皮质醇、血糖、血清溶菌酶(LSZ)、补体C3、超氧化物歧化酶(SOD)和谷胱甘肽(GSH)含量均呈先升高后下降的趋势, 其最大值出现在胁迫2 h或者8 h。在整个胁迫期间, I_0 组皮质醇和血糖含量高于其他各饲料组, I_0 组溶菌酶、补体C3、SOD和GSH均低于其他各饲料组。研究表明, 在低鱼粉饲料中添加牛磺酸可提高青鱼生长性能、改善肠道结构、增强青鱼的抗急性拥挤胁迫的能力。

关键词: 青鱼; 牛磺酸; 低鱼粉; 急性拥挤胁迫; 肠道修复

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鱼粉是水产动物优质蛋白源, 由于其资源紧缺、价格昂贵, 因此, 降低饲料中鱼粉的使用成为目前研究的热点。植物蛋白源因其来源广、价格低、蛋白质含量高成为鱼粉的潜在替代蛋白源, 如豆粕^[1-3]、菜粕^[4-5]、棉籽粕^[6-7]等已在水产饲料中广泛应用, 但由于植物蛋白源含有抗营养因子、适口性差、氨基酸不平衡等原因, 高替代易造成鱼体生长、免疫力和抗应激能力下降, 肠道组织损伤^[8]等负面影响。

牛磺酸(taurine)又称牛胆碱、牛胆素、牛胆

酸, 广泛存在于动物组织细胞内, 鱼粉中有较高含量, 而在植物蛋白中几乎没有^[9]。相关研究表明, 牛磺酸具有诱食^[10]、促进生长和代谢^[11-12]、增强机体免疫力^[13]、提高抗缺氧功能^[14]、修复肌肉损伤^[15]等作用。近期在一些海水鱼中研究表明, 低鱼粉饲料中添加牛磺酸可以促进生长、缓解病症等作用^[16]。

青鱼(*Mylopharyngodon piceus*), 是“四大家鱼”之一, 主要养殖区域为江苏、安徽、湖北和湖南等。毛盼等^[17]用豆粕替代鱼粉研究表明,

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当青鱼幼鱼配合饲料中鱼粉含量低于20%时, 生长速度下降, 饲料系数上升。本实验以20%鱼粉为对照组, 10%的低鱼粉组为负对照, 探讨低鱼粉饲料中添加牛磺酸对缓解低鱼粉、高植物蛋白引起的青鱼生长下降、免疫力降低、肠道受损的负面影响, 为牛磺酸在饲料中的应用提供理论基础和技术支撑。

1 材料与方法

1.1 饲料配方和制备

以青鱼幼鱼实用饲料为基础, 设置正对照组(鱼粉20%), 负对照组(鱼粉10%), 4个实验组: 在低鱼粉(鱼粉10%)饲料中分别添加牛磺酸0.05%、0.1%、0.2%、0.4%(表1), 总共制备6种饲料。饲料制备之前, 鱼粉和豆粕等饲料原料经先粉碎, 过40目筛网, 再按配比从小到大逐级

表1 基础饲料组成及营养水平

Tab. 1 Composition of the basal diet and nutrition level

组分 ingredients	含量 content %					
	对照组	I ₀	I _{0.05}	I _{0.1}	I _{0.2}	I _{0.4}
鱼粉 fish meal	20	10	10	10	10	10
豆粕 soybean meal	24	32	32	32	32	32
棉粕 cottonseed meal	12	16	16	16	16	16
菜粕 rapeseed meal	8	10	10	10	10	10
米糠 rice bran	5	5	5	5	5	5
次粉 middlings	26.46	21.46	21.41	21.36	21.26	21.06
鱼油 fish oil	1.5	2.5	2.5	2.5	2.5	2.5
胆碱 choline	0.5	0.5	0.5	0.5	0.5	0.5
磷酸二氢钙 Ca(H ₂ PO ₄) ₂	1.5	1.5	1.5	1.5	1.5	1.5
预混料 premix	1	1	1	1	1	1
牛磺酸 taurine	0	0	0.05	0.1	0.2	0.4
抗氧化剂 antioxidants	0.01	0.01	0.01	0.01	0.01	0.01
防霉剂 antisepic	0.03	0.03	0.03	0.03	0.03	0.03
营养组成 nutrient composition						
粗蛋白 crude protein	37.36	37.35	37.35	37.34	37.32	37.29
粗脂肪 crude fat	4.85	4.97	4.97	4.96	4.96	4.96
粗灰分 ash	6.88	6.06	6.05	6.05	6.05	6.05
牛磺酸 taurine	0.14	0.08	0.12	0.17	0.27	0.47

定量均匀混合; 再将其进入V型搅拌机充分混合25 min。随后将鱼油与已混好的干粉充分混匀, 再加入适量的水揉匀, 将其加工制成1.5 mm直径的颗粒饲料, 晾干至饲料水分含量为10%左右, 置于-20 °C冰箱中保存备用。

1.2 实验养殖和样品采集

同一规格的同批次健康青鱼鱼苗从湖南省水产科学研究所国家良种场购买, 然后在湖南农业大学实验基地池塘中用商品饲料培育28 d。正式养殖实验前, 桶内驯食一周以适应环境。饥饿24 h后, 挑选规格一致的青鱼幼鱼随机分到18个桶(300 L, 30尾/桶), 青鱼初始体质量为(5.90±0.03) g。每天投喂2次(8:00~9:00, 16:00~17:00), 日投饵量为体质量的3%~5%, 养殖周期为8周。每日换水1次, 换水量占总体积的1/3, 并清除桶内粪便, 日充气12 h以上, 保证溶解氧5.0 mg/L以上, 实验期间水温(28±3) °C, pH为7.31±0.4。养殖实验结束后, 根据生长情况, 选择有代表性的4组, 对照组、未添加牛磺酸组(I₀)、生长最好组(I_{0.1})和牛磺酸高水平添加组(I_{0.4}), 参照明建华等^[18]和Ganga等^[19]的方法, 进行急性拥挤胁迫实验, 其急性拥挤胁迫密度设置为100 g/L。每组设置3个重复。具体过程如下: 实验开始前, 每组每个桶选取15尾规格基本一致的鱼分别转移到直径为25 cm的塑料桶中, 总共36个小桶, 每桶加入1 L水, 使其密度100 g/L, 进行连续24 h拥挤胁迫。实验期间保持正常的水温、溶氧, 试验期间不投饵、减少人为干扰、保持安静、防止额外应激。

养殖实验结束时, 饥饿24 h, 进行称重和计数。每个桶随机取3尾鱼, 冰盘解剖, 用冷冻的去离子水剔除肠周围脂肪和内容物, 取前肠(第一个转折前部分)放入4%甲醛中固定, 编号并标记。苏木精—伊红(H.E)染色制成切片。实验青鱼在急性拥挤胁迫前(0 h)和胁迫中2、8、24 h进行采样。每次取5尾鱼, 用丁香油麻醉, 利用1 mL注射器采取尾静脉取血。血样在4 °C静置12 h后, 以3500 r/min离心10 min制备血清, 放入超低温冰箱(-80 °C)保存备用。

1.3 化学分析

青鱼幼鱼前肠制成切片后, 用LeicaMD 4000B显微镜观察并拍照, 每张切片观察3个视野, 图像由Motic Images Plus 6.0软件进行测量和计算(绒

毛高度、隐窝深度、杯状细胞数、淋巴细胞数), 分别取其平均值作为该样品各指标的测定结果。

1.4 生化分析

血糖(GLU)、超氧化物歧化酶(SOD)、谷胱甘肽(GSH)、溶菌酶(LSZ)分别采用葡萄糖氧化酶—过氧化物酶法、羟胺法、分光光度法、比浊法进行测定, 试剂盒购于南京建成生物有限公司; 补体C3采用免疫比浊法进行测定, 试剂盒购于浙江伊利康公司; 皮质醇采用Elisa鱼类试剂盒; 所有指标均用酶标仪(型号Thermo-1510)检测。

1.5 计算方法和统计分析

实验起始和结束时, 分别对各桶中青鱼进行计数、称重(初重, initial body weight, IBW); 结束时, 从各桶随机选取5尾测体长, 称体质量(末重, final body weight, FBW)、肝重、内脏重。存活率、特定生长率、肥满度、肝体比、脏体比, 计算方法如下:

$$\text{存活率}(\text{survival rate, SR, \%}) = 100 \times W_f/W_i$$

$$\text{增重率}(\text{weight gain rate, WGR, \%}) = 100 \times (W_f - W_0)/W_0$$

$$\text{肥满度}(\text{condition factor, CF, g/cm}^3) = W_f \times 100/L^3$$

$$\text{肝体比}(\text{hepatosomatic index, HSI, \%}) = W_1/W_f \times 100$$

$$\text{脏体比}(\text{ratio of viscera and body weight, VR, \%}) = W_2/W_f \times 100$$

$$W_2/W_f \times 100$$

式中, W_f 为桶内最终鱼数量, W_i 桶内最初鱼数量; $W_0(\text{g})$ 为平均每尾初始体质量; $W_f(\text{g})$ 为平均每尾终末体质量; $W_1(\text{g})$ 为每尾肝脏体质量; $W_2(\text{g})$ 为每尾内脏体质量; $L(\text{cm})$ 为鱼体体长。

实验数据用SPSS 17.0软件进行分析, 当差异显著时($P < 0.05$), 则采用Duncan氏法进行多重比较, 结果用mean±SD表示。

2 结果

2.1 牛磺酸对青鱼幼鱼生长性能的影响

各饲料组间青鱼幼鱼存活率差异不显著。与对照组(20%鱼粉)相比, 低鱼粉饲料(I_0 , 10%鱼粉)组青鱼幼鱼增重率显著降低($P < 0.05$), 随低鱼粉饲料中牛磺酸水平升高, 青鱼增重率呈先上升后下降趋势, 且 $I_{0.1}$ 组增重率显著高于 I_0 组($P < 0.05$), 并与对照组组间差异不显著。相对于对照组, 低鱼粉 I_0 组青鱼幼鱼的肝体比显著增加($P < 0.05$), 低鱼粉饲料中添加牛磺酸有使肝体比下降的趋势, 其中 $I_{0.1}$ 组、 $I_{0.2}$ 组和 $I_{0.4}$ 组肝体比显著低于 I_0 组($P < 0.05$), 但仍显著高于对照组($P < 0.05$)。饲料中牛磺酸在0.0%~0.4%范围内并未显著影响青鱼幼鱼脏体比和肥满度(表2)。

表 2 不同浓度牛磺酸对青鱼幼鱼生长性能的影响

Tab. 2 Effects of taurine on growth performance of juvenile black carp

n=3

组别 group	初重/g IBW	末重/g FBW	增重率/% WGR	成活率/% SR	肥满度/(g/cm ³) CF	脏体比/% VR	肝体比/% HSI
对照组 control group	5.92±0.00	19.48±0.30 ^c	229.05±9.45 ^c	96.67±4.71	1.62±0.04	9.66±0.71	1.50±0.17 ^a
I_0	5.95±0.03	15.21±0.38 ^a	155.63±8.12 ^a	95.00±2.36	1.64±0.07	10.33±0.85	2.18±0.09 ^c
$I_{0.05}$	5.94±0.02	15.68±1.01 ^a	163.97±6.43 ^a	96.67±4.71	1.64±0.05	10.35±0.34	2.27±0.07 ^c
$I_{0.1}$	5.91±0.05	18.94±0.49 ^c	220.47±10.98 ^c	100.00±0.00	1.64±0.07	9.64±0.04	1.79±0.11 ^b
$I_{0.2}$	5.96±0.03	17.23±0.14 ^b	189.09±9.55 ^b	100.00±0.00	1.60±0.04	9.62±0.41	1.84±0.09 ^b
$I_{0.4}$	5.92±0.05	17.04±0.05 ^b	187.84±8.38 ^b	97.78±3.85	1.59±0.05	9.54±0.38	1.86±0.10 ^b

注: 同一列不同字母表示存在显著差异($P < 0.05$), 下同

Notes: Values with the different letters mean significant difference ($P < 0.05$), the same below

2.2 牛磺酸对青鱼幼鱼前肠结构的影响

相对于对照组, 低鱼粉饲料(I_0 组)使青鱼幼鱼前肠绒毛高度显著缩短($P < 0.05$), 低鱼粉饲料中添加牛磺酸使青鱼绒毛高度显著升高($P < 0.05$), 且与对照组差异不显著(表3)。相对于对照组, 低鱼粉饲料(I_0 组)有使青鱼幼鱼前肠隐窝深度变深的趋势, 但差异不显著($P > 0.05$)。低鱼粉饲料中添加牛磺酸使青鱼前肠隐窝深度显著降低($P < 0.05$)。

相对于对照组, 低鱼粉(I_0 组)的青鱼幼鱼杯状细胞数显著减少($P < 0.05$), 淋巴细胞数有减少的趋势。低鱼粉饲料中添加牛磺酸有使青鱼前肠杯状细胞数、淋巴细胞数显著增多的趋势($P < 0.05$)。

2.3 牛磺酸对青鱼幼鱼拥挤胁迫下血液生理生化指标的影响

拥挤胁迫前, I_0 组青鱼血清皮质醇和血糖

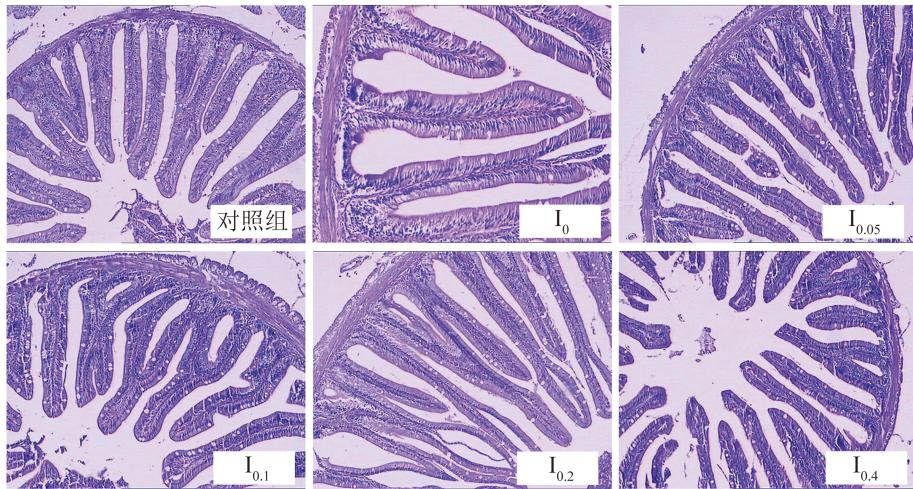


图1 不同浓度牛磺酸对青鱼幼鱼前肠结构的影响(40×)

Fig. 1 Effects of taurine on the structure of anterior intestine in juvenile black carp (40×)

表3 不同浓度牛磺酸对青鱼幼鱼前肠结构的影响

Tab. 3 Effects of taurine on the structure of anterior intestine in juvenile black carp

n=3

组别 group	绒毛高度/ μm villus height	隐窝深度/ μm crypt depth	杯状细胞数/(个/绒毛) goblet cell count	淋巴细胞数/(个/绒毛) lymphocytes count
对照组 control group	469.00±21.40 ^b	249.9±4.50 ^c	10.00±1.00 ^b	83.67±5.51 ^a
I ₀	381.13±14.90 ^a	268.1±9.62 ^c	5.00±1.00 ^a	73.00±2.00 ^a
I _{0.05}	494.66±14.05 ^b	230.7±8.96 ^b	11.00±2.00 ^{bc}	95.00±4.00 ^c
I _{0.1}	480.96±19.10 ^b	256.3±6.52 ^c	18.33±1.53 ^d	89.00±2.00 ^{bc}
I _{0.2}	496.90±3.87 ^b	229.1±15.24 ^b	10.00±1.00 ^b	88.33±2.52 ^{bc}
I _{0.4}	463.95±24.25 ^b	185.4±4.30 ^a	12.67±1.53 ^c	86.67±4.51 ^b

(GLU)含量显著高于对照组($P<0.05$)，添加牛磺酸使血清皮质醇和GLU含量显著降低($P<0.05$)，与对照组无显著差异；I₀组青鱼血清超氧化物歧化酶(SOD)活力、溶菌酶(LSZ)活力显著低于对照组($P<0.05$)，添加牛磺酸使血清SOD活力和LSZ活力显著提高($P<0.05$)，且I_{0.1}、I_{0.2}、I_{0.4}组青鱼LSI、I_{0.1}组血清SOD活力与对照组无显著差异；I₀组青鱼血清谷胱甘肽(GSH)和补体C3含量与对照组无显著差异，添加牛磺酸使青鱼血清补体C3、GSH含量显著提高($P<0.05$)，其中牛磺酸组血清GSH含量与对照组差异不显著，而补体C3含量显著高于低鱼粉组(I₀组)和对照组($P<0.05$)(图2)。

拥挤胁迫(0~24 h)使青鱼幼鱼血清各指标均呈先升高后下降的趋势，血清GLU、GSH含量及SOD活力在8 h达到最大值，血清LSZ活性和补体C3含量在2 h达到最大值。I₀组青鱼血清皮质醇含

量最大值出现在8 h，其他饲料组青鱼血清皮质醇含量在2 h达到最大值。在整个胁迫期间，低鱼粉饲料(I₀组)组青鱼血清GLU和皮质醇含量均显著高于其他各饲料组($P<0.05$)，而血清SOD活力、LSZ活性、GSH和补体C3含量显著低于其他各饲料组，但低鱼粉饲料添加牛磺酸使青鱼血清SOD活力、LSZ活性、GSH和补体C3含量显著提高($P<0.05$)。拥挤胁迫24 h后，除了血清补体C3和I₀组皮质醇含量外，青鱼血清中其他各项指标恢复到胁迫前(0 h)水平。

3 讨论

本实验表明，低鱼粉饲料使青鱼幼鱼前肠绒毛高度显著变短，隐窝深度显著变深，杯状细胞数和淋巴细胞数变少，与谷琨^[8]在海水鱼虾上的研究结果相似。前肠是鱼类营养消化吸收的重要场所，肠道的组织形态发育与动物的生

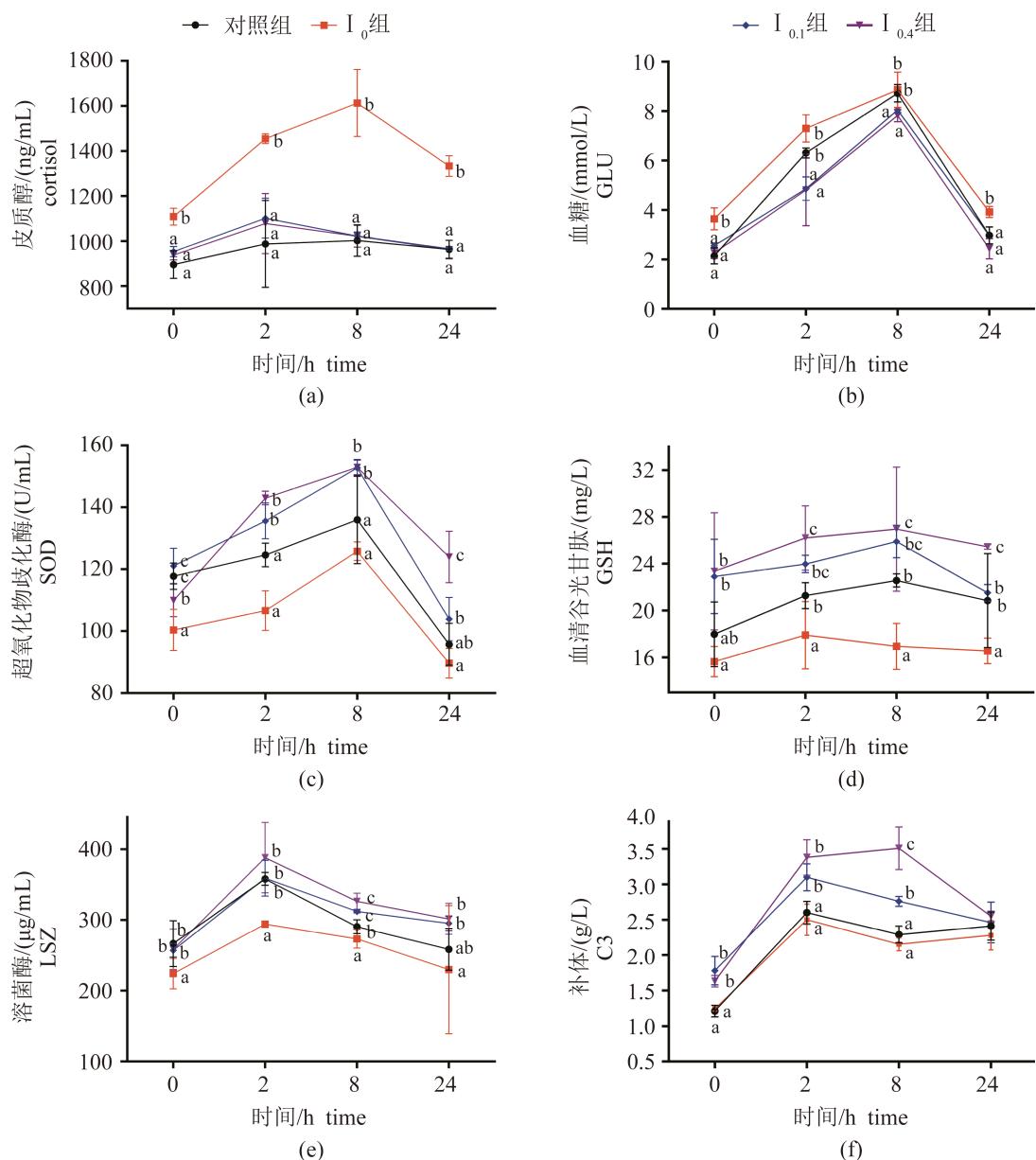


图 2 牛磺酸对急性拥挤胁迫青鱼血清指标的影响

字母不同代表差异显著($P<0.05$)

Fig. 2 Effects of taurine levels on serum in juvenile black carp to acute crowding stress

The different letters mean significant difference ($P<0.05$)

长和营养物质的消化吸收有重要的关系。小肠绒毛的主要功能是吸收营养物质，绒毛高度的增加可以使小肠吸收养分的面积增大，因此绒毛高度直接影响动物的生长发育。隐窝深度反映肠上皮细胞更新速度，隐窝变浅表明肠上皮细胞生成率降低、更新过程减慢而成熟率上升，分泌吸收功能增强。杯状细胞数可分泌黏蛋白、三叶肽等物质，有保护肠道黏膜的作用。绒毛高度、隐窝深度、杯状细胞数、淋巴

细胞数可作为肠道发育和损伤的标志^[20-21]。低鱼粉饲料使青鱼生长性能显著下降，与抗营养因子通过损伤肠道绒毛，使杯状细胞数和淋巴细胞数减少来降低营养物质的利用率有关^[22]。而低鱼粉饲料添加牛磺酸可使青鱼幼鱼绒毛高度增加，隐窝深度变浅，杯状细胞数和淋巴细胞数增多，说明牛磺酸可以通过修复肠道损伤来提高营养物质的吸收利用来促生长^[8]。牛磺酸对青鱼促生长效果，与牛磺酸与在大麻哈鱼(*Oncor-*

hynchus keta)^[22]、黄尾鱥(*Seriola quinqueradiata*)^[23]、尼罗罗非鱼(*Oreochromis niloticus*)^[24]的研究结果类似。原因可能是牛磺酸具有刺激性气味, 适量的添加对机体有诱食作用, 提高摄食率; 也有可能是牛磺酸的添加可抑制机体蛋氨酸、半胱氨酸、胱氨酸参与合成牛磺酸, 从而使其更多地参与蛋白质合成^[25-26]。但当牛磺酸的添加量超过0.1%时, 青鱼增重率呈下降趋势, 可能与牛磺酸具有酸味, 过量的牛磺酸使饲料的适口性变差, 摄食率下降有关^[27]。但牛磺酸添加效果与鱼类发育阶段、鱼类自身合成牛磺酸的能力高低、饲料中鱼粉高低相关。如饲料中添加1.5%牛磺酸能显著提高大菱鲆(*Scophthalmus maximus*)(初重 6.31 ± 0.01 g)的生长, 但对初重(165.9 ± 5.01 g)大菱鲆的生长无明显影响^[28]。牛磺酸能显著提高摄食植物蛋白的虹鳟(*Oncorhynchus mykiss*)的增重率, 但对高鱼粉组无显著影响^[29]。大太阳鱼(*Lepomism macrochirus*)和真鲷(*Pagrus major*)肝脏在牛磺酸能力合成能力存在较大的差异^[30-32]。

恒定的血糖水平对维持鱼类正常生命活动起着重要作用。皮质醇是一种糖皮质激素, 对鱼类的营养代谢具有调控作用, 还可以增强与糖异生有关酶的活性, 促进血糖含量升高, 是反映鱼类应激的灵敏信号^[33]。研究显示, 当机体受到应激时, 需消耗更多的能量来缓解胁迫引起的不适, 会引起皮质醇和血糖含量升高。本实验结果表明, 拥挤胁迫使低鱼粉饲料青鱼血清皮质醇和血糖含量显著高于高鱼粉对照组, 而牛磺酸可缓减急性拥挤胁迫引起的血清皮质醇和血糖含量升高, 与团头鲂(*Megalobrama amblycephala*)^[18]、黄斑蓝子鱼(*Siganus canaliculatus*)^[34]浅水应激结果类似, 说明在低鱼粉饲料中添加适量牛磺酸可以缓减高植物蛋白引起的抗急性拥挤胁迫能力下降的负面影响。拥挤胁迫24 h后, 高鱼粉组和牛磺酸组血清皮质醇和血糖含量基本恢复到应激前(0 h)水平, 但低鱼粉组皮质醇仍然高于应激前(0 h)水平, 说明机体具有自我调整适应环境变化的能力, 且牛磺酸可以缩短机体对外界应激的适应时间。

鱼类不具有健全的免疫机制, 非特异免疫功能在鱼类防御中属于第一道防线。溶菌酶和补体C3是非特异性免疫机制中重要的组成部分。低鱼粉饲料使青鱼幼鱼溶菌酶活性显著降

低, 添加牛磺酸后, 青鱼幼鱼补体C3含量和溶菌酶活性升高, 说明低鱼粉饲料中添加牛磺酸使青鱼幼鱼非特异性免疫功能增强, 与在鲤(*Cyprinus carpio*)上研究结果一致^[35]。短时间(0~2 h)急性拥挤胁迫使青鱼幼鱼血清补体C3含量和溶菌酶活性升高, 说明机体具有通过提高血清非特异性免疫来防御疾病和急性环境胁迫的能力^[36-37]。低鱼粉饲料中添加牛磺酸后, 其血清补体C3含量和溶菌酶活性比低鱼粉饲料组升高幅度更大, 说明牛磺酸可以通过提高鱼类机体的非特异免疫力来抵抗急性胁迫带来的不适^[37]。

机体拥有一套完整的酶(如: SOD)和非酶抗氧化系统(如: GSH), 抗氧化酶活性的高低能够反映机体清除自由基的能力^[38]。低鱼粉饲料使青鱼幼鱼血清SOD活力显著降低, 但添加牛磺酸可使血清GSH含量和SOD活力提高, 说明牛磺酸可缓解由高植物蛋白引起的机体自由基升高, 脂质过氧化增加, 抗氧化力下降的现象, 这与在建鲤幼鱼(*Cyprinus carpio var. jian*)上的研究结果一致^[39]。短时间(0~2 h)急性拥挤胁迫使青鱼幼鱼血清GSH含量和SOD活力升高, 与团头鲂结果类似^[18], 表明机体具有通过提高血清GSH含量和SOD活力来清除急性拥挤胁迫下机体产生自由基的能力。急性拥挤胁迫下牛磺酸饲料组的青鱼幼鱼有更高的血清GSH含量和SOD活力, 说明牛磺酸有利于机体抵抗外界的应激, 能使机体更快维持稳态, 其机理还有待进一步研究。

4 结论

在本实验条件下, 低鱼粉饲料中添加牛磺酸有效提高青鱼幼鱼生长性能、改善肠道结构, 提高抗急性拥挤胁迫能力, 且添加量为0.1%时可达到较好的效果。

参考文献:

- [1] 向枭, 周兴华, 陈建, 等. 饲料中豆粕蛋白替代鱼粉蛋白对齐口裂腹鱼幼鱼生长性能、体成分及血液生化指标的影响[J]. 水产学报, 2012, 36(5): 723-731.
Xiang X, Zhou X H, Chen J, et al. Effect of dietary replacement of fish meal protein with soybean meal protein on the growth, body composition and hematology indices of *Schizothorax prenanti*[J]. Journal of Fisheries of China, 2012, 36(5): 723-731 (in Chinese).

- [2] 姜俊, 胡肆, 周小秋, 等. 膨化饲料中豆粕替代鱼粉比例对建鲤肌肉品质的影响[J]. 动物营养学报, 2015, 27(2): 623-630.
- Jiang J, Hu Y, Zhou X Q, et al. Effects of replacement ratio of fish meal by soybean meal in extruded diets on muscle quality of Jian Carp (*Cyprinus carpio* var. *jian*)[J]. Chinese Journal of Animal Nutrition, 2015, 27(2): 623-630 (in Chinese).
- [3] Wu Y, Han H, Qin J, et al. Replacement of fishmeal by soy protein concentrate with taurine supplementation in diets for golden pompano (*Trachinotus ovatus*)[J]. Aquaculture Nutrition, 2015, 21(2): 214-222.
- [4] 张杰, 王四维, 陈泽涛, 等. 菜籽粕替代鱼粉对银鲫生长性能及饲料利用率的影响[J]. 中国粮油学报, 2013, 28(9): 91-96.
- Zhang J, Wang S W, Chen Z T, et al. Effect of substituting fish meal with rapeseed meal on the growth and feed utilization of *Carassius auratus*[J]. Journal of the Chinese Cereals and Oils Association, 2013, 28(9): 91-96 (in Chinese).
- [5] Slawski H, Adem H, Tressel R P, et al. Replacement of fishmeal by rapeseed protein concentrate in diets for Common Carp (*Cyprinus carpio* L.)[J]. The Israeli Journal of Aquaculture-Bamidgeh, 2011, 63: 605.
- [6] 严全根, 朱晓鸣, 杨云霞, 等. 饲料中棉粕替代鱼粉蛋白对草鱼的生长、血液生理指标和鱼体组成的影响[J]. 水生生物学报, 2014, 38(2): 362-369.
- Yan Q G, Zhu X M, Yang Y M, et al. Effect of replacement of fish meal with cottonseed meal on growth, hematological physiology, and body composition of grass carp[J]. Acta Hydrobiologica Sinica, 2014, 38(2): 362-369 (in Chinese).
- [7] Pham M A, Lee K J, Lim S J, et al. Evaluation of cottonseed and soybean meal as partial replacement for fishmeal in diets for juvenile Japanese flounder *Paralichthys olivaceus*[J]. Fisheries Science, 2007, 73(4): 760-769.
- [8] 谷珉. 影响海水鱼虾对植物蛋白利用的抗营养因子和蛋氨酸的研究[D]. 青岛: 中国海洋大学, 2013.
- Gu M. The research on the plant protein utilization by marine fish and shrimp-antinutritional factors and methionine[D]. Qingdao: Ocean University of China, 2013 (in Chinese).
- [9] 王和伟, 叶继丹, 陈建春. 牛磺酸在鱼类营养中的作用及其在鱼类饲料中的应用[J]. 动物营养学报, 2013, 25(7): 1418-1428.
- Wang H W, Ye J D, Chen J C. Taurine: Effect in fish nutrition and application in fish feed[J]. Chinese Journal of Animal Nutrition, 2013, 25(7): 1418-1428 (in Chinese).
- [10] Døving K B, Selset R, Thommesen G. Olfactory sensitivity to bile acids in salmonid fishes[J]. Acta Physiologica Scandinavica, 1980, 108(2): 123-131.
- [11] López L M, Flores-Ibarra M, Bañuelos-Vargas I, et al. Effect of fishmeal replacement by soy protein concentrate with taurine supplementation on growth performance, hematological and biochemical status, and liver histology of totoaba juveniles (*Totoaba macdonaldi*)[J]. Fish Physiology and Biochemistry, 2015, 41(4): 921-936.
- [12] Johnson R B, Kim S K, Watson A M, et al. Effects of dietary taurine supplementation on growth, feed efficiency, and nutrient composition of juvenile sablefish (*Anoplopoma fimbria*) fed plant based feeds[J]. Aquaculture, 2015, 445: 79-85.
- [13] 凌云. 牛磺酸对虎纹蛙(*Hoplobatrachus rugulosus*)非特异性免疫、消化酶活力及抗氧化能力的影响[D]. 金华: 浙江师范大学, 2012.
- Ling Y. The effect of taurine on the nonspecific immune response, digestive enzyme activity and antioxidant ability in the tiger frogs (*Hoplobatrachus rugulosus*)[D]. Jinhua: Zhejiang Normal University, 2012 (in Chinese).
- [14] Yang H J, Tian L X, Huang J W, et al. Dietary taurine can improve the hypoxia-tolerance but not the growth performance in juvenile grass carp *Ctenopharyngodon idellus*[J]. Fish Physiology and Biochemistry, 2013, 39(5): 1071-1078.
- [15] Pierno S, Liantonio A, Camerino G M, et al. Potential benefits of taurine in the prevention of skeletal muscle impairment induced by disuse in the hindlimb-unloaded rat[J]. Amino Acids, 2012, 43(1): 431-445.
- [16] Takagi S, Murata H, Goto T, et al. Taurine is an essential nutrient for yellowtail *Seriola quinqueradiata* fed non-fish meal diets based on soy protein concentrate[J]. Aquaculture, 2008, 280(1-4): 198-205.
- [17] 毛盼, 胡毅, 李金龙, 等. 豆粕替代鱼粉对青鱼幼鱼生长及生理生化指标的影响[J]. 淡水渔业, 2013, 43(5): 50-56, 67.

- Mao P, Hu Y, LI J L, et al. Effects of fish meal replacement by dietary soybean meal levels on growth and biochemical indices in juvenile *Mylopharyngodon piceus*[J]. Freshwater Fisheries, 2013, 43(5): 50-56, 67 (in Chinese).
- [18] 明建华, 谢骏, 徐跑, 等. 大黄素、维生素C及其配伍对团头鲂抗拥挤胁迫的影响[J]. 水生生物学报, 2011, 35(3): 400-413.
- Ming J H, Xie J, Xu P, et al. Effects of emodin, vitamin C and their combination on crowding stress resistance of Wuchang Bream (*Megalobrama amblycephala* Yih)[J]. Acta Hydrobiologica Sinica, 2011, 35(3): 400-413 (in Chinese).
- [19] Ganga R, Montero D, Bell J G, et al. Stress response in sea bream (*Sparus aurata*) held under crowded conditions and fed diets containing linseed and/or soybean oil[J]. Aquaculture, 2011, 311(1-4): 215-223.
- [20] 林谦, 戴求仲, 宾石玉, 等. 饲粮添加益生菌与酶制剂对黄羽肉鸡生长性能的影响及相关机理[J]. 动物营养学报, 2012, 24(10): 1955-1965.
- Lin Q, Dai Q Z, Bin S Y, et al. Probiotics and enzyme preparation: Effects on growth performance of Yellow-feathered broilers and its mechanism[J]. Chinese Journal of Animal Nutrition, 2012, 24(10): 1955-1965 (in Chinese).
- [21] 隋欣. 益生菌对雏鸡肠道杯状细胞数量及黏蛋白2含量的影响[D]. 哈尔滨: 东北农业大学, 2014.
- Sui X. Influence of the number of goblet cells and the content of MUC2 in intestinal tract of chicks after the application of probiotics[D]. Harbin: Northeast Agricultural University, 2014 (in Chinese).
- [22] Krogdahl Å, Penn M, Thorsen J, et al. Important antinutrients in plant feedstuffs for aquaculture: An update on recent findings regarding responses in salmonids[J]. Aquaculture Research, 2010, 41(3): 333-344.
- [23] Nguyen H P, Khaoian P, Fukada H, et al. Feeding fermented soybean meal diet supplemented with taurine to yellowtail *Seriola quinqueradiata* affects growth performance and lipid digestion[J]. Aquaculture Research, 2015, 46(5): 1101-1110.
- [24] 周铭文, 王和伟, 叶继丹. 饲料牛磺酸对尼罗罗非鱼生长、体成分及组织游离氨基酸含量的影响[J]. 水产学报, 2015, 39(2): 213-223.
- Zhou M W, Wang H W, Ye J D. Effects of taurine supplementation on the growth, body composition and tissue free amino acid concentrations in Nile tilapia (*Oreochromis niloticus*)[J]. Journal of Fisheries of China, 2015, 39(2): 213-223 (in Chinese).
- [25] Chatzifotis S, Polemitou I, Divanach P, et al. Effect of dietary taurine supplementation on growth performance and bile salt activated lipase activity of common dentex, *Dentex dentex*, fed a fish meal/soy protein concentrate-based diet[J]. Aquaculture, 2008, 275(1-4): 201-208.
- [26] Matsunari H, Furuita H, Yamamoto T, et al. Effect of dietary taurine and cystine on growth performance of juvenile red sea bream *Pagrus major*[J]. Aquaculture, 2008, 274(1): 142-147.
- [27] 罗莉, 文华, 王琳, 等. 牛磺酸对草鱼生长、品质、消化酶和代谢酶活性的影响[J]. 动物营养学报, 2006, 18(3): 166-171.
- Luo L, Wen H, Wang L, et al. Effects of taurine on growth performance, quality, digestive and metabolic enzyme activity of Grass Carp (*Ctenopharyngodon idellus*)[J]. Chinese Journal of Animal Nutrition, 2006, 18(3): 166-171 (in Chinese).
- [28] Qi G S, Ai Q H, Mai K S, et al. Effects of dietary taurine supplementation to a casein-based diet on growth performance and taurine distribution in two sizes of juvenile turbot (*Scophthalmus maximus* L.)[J]. Aquaculture, 2012, 358-359: 122-128.
- [29] Gaylord T G, Teague A M, Barrows F T. Taurine supplementation of all-plant protein diets for rainbow trout (*Oncorhynchus mykiss*)[J]. Journal of the World Aquaculture Society, 2006, 37(4): 509-517.
- [30] Goto T, Matsumoto T, Murakami S, et al. Conversion of cysteate into taurine in liver of fish[J]. Fisheries Science, 2003, 69(1): 216-218.
- [31] Goto T, Tiba K, Sakurada Y, et al. Determination of hepatic cysteinesulfinate decarboxylase activity in fish by means of OPA-prelabeling and reverse-phase high-performance liquid chromatographic separation[J]. Fisheries Science, 2001, 67(3): 553-555.
- [32] Goto T, Matsumoto T, Takagi S. Distribution of the hepatic cysteamine dioxygenase activities in fish[J]. Fisheries Science, 2001, 67(6): 1187-1189.
- [33] 丁厚猛. 养殖密度对杂交幼鱼摄食、生长及生理机能的影响[D]. 青岛: 中国海洋大学, 2014.

- Ding H M. Effect of stocking density on feeding and growth and physiological function of juvenile hybrid sturgeon[D]. Qingdao: Ocean University China, 2014 (in Chinese).
- [34] 卢玉标, 游翠红, 王树启, 等. 浅水应激后黄斑蓝子鱼生理指标变化及牛磺酸的抗应激作用[J]. 水生生物学报, 2014, 38(1): 68-74.
- Lu Y B, You C H, Wang S Q, et al. Physiological changes in *Siganus canaliculatus* after shallow water stress and the anti-stress effects of taurine[J]. Acta Hydrobiologica Sinica, 2014, 38(1): 68-74 (in Chinese).
- [35] 邱小琼, 赵红雪, 王远吉, 等. 牛磺酸对鲤非特异性免疫及抗氧化能力的影响[J]. 上海水产大学学报, 2008, 17(4): 429-434.
- Qiu X C, Zhao H X, Wang Y J, et al. Effect of taurine on the non-specific immunity and antioxidative competence of carp[J]. Journal of Shanghai Fisheries University, 2008, 17(4): 429-434 (in Chinese).
- [36] Møyner K, Røed K H, Sevatdal S, et al. Changes in non-specific immune parameters in Atlantic salmon, *Salmo salar* L., induced by *Aeromonas salmonicida* infection[J]. Fish & Shellfish Immunology, 1993, 3(4): 253-265.
- [37] Demers N E, Bayne C J. The immediate effects of stress on hormones and plasma lysozyme in rainbow trout[J]. Developmental & Comparative Immunology, 1997, 21(4): 363-373.
- [38] 扈添琴, 韩兆玉, 王群, 等. 酶制剂和植物甾醇复合物对泌乳奶牛生产性能和血清指标的影响[J]. 动物营养学报, 2014, 26(1): 236-244.
- Hu T Q, Han Z Y, Wang Q, et al. Effects of enzyme-phytosterol complex on performance and serum indices of lactating dairy cows[J]. Chinese Journal of Animal Nutrition, 2014, 26(1): 236-244 (in Chinese).
- [39] Feng L, Zhao B, Chen G F, et al. Effects of dietary histidine on antioxidant capacity in juvenile Jian carp (*Cyprinus carpio* var. *jian*)[J]. Fish Physiology and Biochemistry, 2013, 39(3): 559-571.

Effect of dietary taurine supplementation on growth, intestine structure and resistance to acute crowding stress in juvenile black carp (*Mylopharyngodon piceus*) fed low fish meal diets

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Abstract: An 8-week feeding experiment was conducted to investigate the effects of dietary graded level of taurine supplementation on growth performance, intestine structure and resistance to acute crowding stress of juvenile black carp (*Mylopharyngodon piceus*) (initial mean body weight 5.90 ± 0.03 g). The diet containing 20% fish meal was set as the control group. All the other groups contained 10% fish meal and were supplemented with 0% (I_0), 0.05% ($I_{0.05}$), 0.1% ($I_{0.1}$), 0.2% ($I_{0.2}$) and 0.4% ($I_{0.4}$) taurine, respectively. Each diet was randomly fed to triplicate groups of 30 fishes per tank (300 L). After the feeding experiment, according to growth performance, a 24 h acute crowding stress was conducted in 4 group (the control, I_0 , $I_{0.1}$, $I_{0.4}$, and the density is 100 g/L). The results showed that the weight gain rate (WGR) of black carp in I_0 group was significantly lower than that in the control group ($P<0.05$). At the lower fish meal level, WGR of black carp first rose and then decreased with increasing dietary taurine supplementation level, and the maximum value was found in 0.1% group ($P<0.05$), who had no significant difference with the control group. Compared with the control, the villus height of the I_0 group were higher, crypt depth and number of goblet cells of the I_0 group were smaller; At the lower fish meal level, all fish fed the diets with dietary taurine supplementation had a greater crypt depth, goblet cell and lymphocytes number. Moreover, a lower villus height, villus height, crypt depth, goblet cell and lymphocytes number of intestine in the $I_{0.1}$ group were significantly higher than the control group ($P<0.05$). As the growth of the crowded stress time, cortisol, blood glucose (GLU) and lysozyme (LSZ), complement C3, superoxide dismutase (SOD) and glutathione (GSH) all had a trend of first rose and then decreased, and the maximum were found at 2 h and 8 h. There were different increase of these indexes among dietary treatments. The serum glucose and cortisol of fish fed the diet with 10% fish meal level were significantly higher than those in the other group ($P<0.05$), but serum LSZ, complement C3, SOD and GSH in I_0 group lower than those in the other group ($P>0.05$). These results suggested that dietary taurine supplementation can enhance growth performance of black carp, improve intestine structure, and increase the ability of black carp's resistance to acute crowding stress.

Key words: *Mylopharyngodon piceus*; taurine; low fish meal; acute crowding stress; intestine structure

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