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精氨酸对许氏平鲉豆粕型肠炎的修复作用研究*

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摘要 为研究精氨酸(Arg)对患有豆粕型肠炎(SBMIE)的许氏平鲉(*Sebastes schlegelii*)生长性能、Arg 代谢、肠道组织结构、抗氧化性能、肠道紧密连接蛋白基因(*occludin*、*clnd15* 和 *zo-1*)及炎症因子基因(*il-1β*、*il-8*、*il-15* 和 *tlr8*)和抗炎因子基因(*il-12b*)表达量的影响，以(54.97±0.12) g 已诱导出 SBMIE 的许氏平鲉为研究对象，基础配方中添加 30%豆粕，以 Arg 0 添加为对照组(D0)，添加 1%、2%和 3% Arg 为处理组(D1、D2 和 D3)，配制 4 组等氮等能的实验饲料，每组 3 个重复，每个重复 40 尾鱼，进行为期 6 周的养殖实验。结果显示，D2 和 D3 组实验鱼增重率显著升高(P<0.05)，各处理组肝体比和脏体比均显著低于 D0 组，肥满度显著高于 D0 组(P<0.05)；处理组血清二胺氧化酶(DAO)、诱导型一氧化氮合成酶(iNOS)活性及一氧化氮(NO)含量显著降低(P<0.05)，D2 和 D3 组总一氧化氮合成酶(T-NOS)活性显著降低(P<0.05)；Arg 显著提高了实验鱼肠道皱襞高度(P<0.05)；处理组肠道总抗氧化能力显著升高，并以 D2 组最高(P<0.05)，D2 和 D3 组丙二醛含量显著降低(P<0.05)；相比较 D0 组，各处理组 *occludin* mRNA 相对表达量显著上调，D2 组 *clnd15* 和 D1 组 *zo-1* mRNA 相对表达量显著上调(P<0.05)；处理组肠道 *il-1β*、*il-15* 和 *tlr8* mRNA 相对表达量显著下调，*il-12b* mRNA 相对表达量显著上调(P<0.05)。综上所述，在本实验条件下，高豆粕饲料中添加 Arg 能显著提高 SBMIE-许氏平鲉的生长性能和抗氧化性能，改善 Arg 代谢和肠道组织结构，上调肠道紧密连接蛋白和抗炎因子基因相对表达量，下调炎症因子基因相对表达量。Arg (2%最佳)对许氏平鲉 SBMIE 具有修复作用。本研究为 Arg 修复 SBMIE 的机制提供了理论依据。

关键词 许氏平鲉；精氨酸；豆粕型肠炎；紧密连接蛋白；炎症因子

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近年来，鱼粉价格不断攀升，配合饲料成本高居不下，为降低饲料成本，豆粕等产量稳定、价格低廉的植物蛋白源在水产饲料中的用量逐渐增加，但由于豆粕中含有大豆凝集素、植酸和皂甙等抗营养因子，过量替代会引起鱼类肠道损伤，诱发豆粕型肠炎(soybean meal-induced enteritis, SBMIE)(Gu *et al*, 2016)。对斜带石斑鱼(*Epinephelus coioides*)(Wang *et al*, 2017)、大菱鲆(*Scophthalmus maximus*)(Gu *et al*,

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2016)、虹鳟(*Oncorhynchus mykiss*)(Merrifield *et al*, 2009)等鱼的研究均报道了豆粕过量替代鱼粉后豆粕型肠炎的发生。为缓解豆粕对鱼类肠道的损伤，提高其在饲料中的利用，Gu 等(2017)验证了谷氨酰胺、精氨酸等功能性氨基酸在肠道修复方面的积极作用。精氨酸(Arginine，Arg)是鱼类的必需氨基酸，又是一种功能性氨基酸，不仅参与蛋白质和嘧啶合成、激素释放，还是合成尿素、谷氨酸、肌酸、多胺和一氧化氮(NO)等生物活性物质的前体(万军利等, 2006)，参与机体生长、免疫、肠道屏障、内分泌等多种代谢调节，在免疫调节及维持和保护肠道黏膜结构和功能等方面起着重要作用(Wang *et al*, 2009)。在畜禽(姜海龙等, 2015)和淡水鱼(Cheng *et al*, 2011; Jiang *et al*, 2015)中均报道了 Arg 有利于肠道黏膜损伤后的修复。陈娇娇(2017)离体培养草鱼(*Ctenopharyngodon idella*)肠道黏膜细胞发现，Arg 能促进草鱼细胞间紧密连接蛋白基因的表达；Jiang 等(2015)对建鲤(*Cyprinus carpiovar*. Jian)的研究发现，Arg 能够调节脂多糖诱导的肠道炎症反应，抑制炎症因子的表达，促进抗炎因子的表达，但作用机制尚不清楚。

许氏平鲉(*Sebastes schlegelii*)是我国北方网箱养殖和增殖放流的理想种类(李宝山等, 2019)，作为肉食性经济鱼类，其对鱼粉需求较高，豆粕等植物蛋白过量添加易损伤肠道健康。本研究的前期实验诱导了许氏平鲉肠炎的发生，实验鱼均出现黏膜皱襞高度降低、细胞核排列紊乱、杯状细胞明显增多等 SBMIE 的典型症状。基于以上实验，本研究以诱导出豆粕型肠炎的许氏平鲉[(54.97±0.12) g]为研究对象，从生长性能、Arg 代谢、肠道组织结构、紧密连接蛋白基因(*occludin*、*clnd15* 和 *zo-1*)、炎症因子基因(*il-1β*、*il-8*、*il-15* 和 *tlr8*)和抗炎因子基因(*il-12b*)表达等多方面探讨 Arg 对豆粕型肠炎的修复作用及其机理，以期为 Arg 维护鱼类肠道健康方面的应用提供科学依据，为植物蛋白在肉食性经济鱼类许氏平鲉配合饲料中的应用提供参考。

1　材料与方法

1.1　实验饲料

以白鱼粉、豆粕和酪蛋白为主要蛋白源，鱼油为主要脂肪源，Arg 0 添加为对照组(D0)，Arg (L 型，纯度 98%，上海麦克林生化科技有限公司)添加 1%、2%和 3%为处理组(D1、D2 和 D3)，用甘氨酸(L 型，纯度 98%，上海麦克林生化科技有限公司)配平饲料总氨基酸水平，配制 4 组等氮等能的实验饲料，饲料配方、营养组成及饲料 Arg 实测值见表 1。

表 1　饲料配方及营养组成

Tab.1　Composition and nutrient levels of the experimental diets/%

原料 Ingredients	组别 Groups			
	D0	D1	D2	D3
白鱼粉 White fish meal	28.00	28.00	28.00	28.00
豆粕 Soybean meal	30.00	30.00	30.00	30.00
酪蛋白 Casein	16.00	16.00	16.00	16.00
精氨酸 Arginine	0	1.00	2.00	3.00
甘氨酸 Glycine	3.00	2.00	1.00	0
谷朊粉 Wheat gluten	2.40	2.40	2.40	2.40
A-淀粉 α-Starch	8.00	8.00	8.00	8.00
鱼油 Fish oil	8.00	8.00	8.00	8.00
维生素预混料 Vitamin premix	1.00	1.00	1.00	1.00
矿物质预混料 Mineral premix	1.00	1.00	1.00	1.00
甜菜碱 Betaine	1.00	1.00	1.00	1.00
大豆卵磷脂 Soybean lecithin	1.00	1.00	1.00	1.00
氯化胆碱 Choline chloride	0.50	0.50	0.50	0.50
抗氧化剂 Antioxidant	0.10	0.10	0.10	0.10
合计 Total	100.00	100.00	100.00	100.00
营养组成 Nutrient composition				
粗蛋白 Crude protein	52.11	52.16	52.32	52.15
粗脂肪 Crude lipid	11.41	11.37	11.22	11.52
粗灰分 Crude ash	10.84	10.78	10.74	11.01
能量 Energy kJ/g	19.97	19.99	19.97	20.60
精氨酸 Arginine	2.80	3.80	4.83	5.82

注：维生素预混料和矿物质预混料配方组成见参考文献(沈钰博等, 2022)。

Note: Same contents of vitamin premix and mineral premix as reference (Shen *et al*, 2022).

1.2　实验动物及饲养管理

养殖实验在山东省海洋资源与环境研究院(东营)试验基地车间的水泥池进行，实验用许氏平鲉购自威海泰丰海水育苗有限公司，选用同一批次的许氏平鲉，预实验设置 30%、40%和 50%的豆粕添加量，分别于实验的第 14、21 和 28 天取样，观察肠道的炎症程度，以所有样品均出现明显的肠道炎症为标准决定豆粕添加量及诱导周期，诱导实验最终使用 40%豆粕饲料喂养 28 d，诱导豆粕型肠炎的发生。修复实验开始前，挑选 480 尾初体重(initial body weight, IBW)为(54.97± 1.12) g、已被诱导出豆粕型肠炎的许氏平鲉，随机分为 4 组，每组 3 个重复，每个重复 40 尾鱼，随机置于 12 个自制网箱(60 cm×60 cm×90 cm)中。修复实验进行 6 周，每天定时(08:00 和 17:00)定量投喂 2 次，初始投喂量为体重的 1%，根据摄食情况调整

投喂量并记录死鱼数量和重量。驯养及实验期间每天清底并加注新水，控制水温为 18~22 ℃，pH 值为 7.6~8.2，溶氧>6 mg/L，氨氮和亚硝酸盐浓度<0.05 mg/L，光照周期为自然光周期。

1.3 样品采集

实验结束后禁食 24 h，每桶鱼计数并称末体重(final body weight, FBW)。每桶随机取 10 尾鱼，麻醉后测量体长和体重，尾静脉采血后分离内脏，所有操作均在冰盘内完成。血液于 4 ℃静置 4 h 后离心(4 000 r/min, 10 min)，血清保存于–20 ℃，用于酶活性及代谢产物测定。每桶取 3 尾鱼中后肠，生理盐水匀浆后离心(2 500 r/min, 10 min)，上清液保存于–20 ℃，用于酶活性测定。每桶取 3 尾鱼后肠(0.8 cm)固定于 Bouin's 液中，24 h 后转移到 70%酒精中，经脱水、透明、浸蜡、包埋后，进行常规石蜡连续切片(厚度 7.0 μm)，HE 染色后，中性树胶封片，徕卡高清摄像系统(LEICAICC50HD)下观察并拍照。每桶取 6 尾鱼后肠(1 cm)于液氮中，转移至–80 ℃冰箱保存，用于基因表达量测定。

1.4 计算公式与实验方法

增重率(weight gain rate, WGR, %)=[FBW(g)–IBW(g)]×100/IBW(g)；

肝体比(hepatosomatic index, HSI, %)=肝脏质量(g)×100/FBW(g)；

脏体比(viscerosomatic index, VSI, %)=内脏质量(g)×100/FBW(g)；

肥满度(condition factor, CF)=FBW(g)×100/体长$(cm)^3$；

存活率(survival rate, SR, %)=成活尾数×100/总尾数。

饲料粗蛋白采用凯氏定氮法(GB/T 6432-2006)测定；粗脂肪采用索氏抽提法(GB/T 6433-2006)测定；粗灰分采用 550 ℃失重法(GB/T 6438-2007)测定；饲料 Arg 使用氨基酸分析仪(HITACHI L-8900)测定。

肠道总抗氧化能力(total antioxidant capacity, T-AOC)、丙二醛(malondialdehyde, MDA)含量，血清二胺氧化酶(diamine oxidase, DAO)、总一氧化氮合酶(T-NOS)、诱导型一氧化氮合酶(iNOS)活性及一氧化氮(NO)含量均采用南京建成生物工程研究所试剂盒测定。

1.5 实时荧光定量 PCR 检测

使用山东思科捷生物技术有限公司试剂盒(SPARKeasy Improved Tissue/Cell RNA Kit)进行总 RNA 提取，NanoDrop®2000 (Thermo Fisher Scientific, 美国)检测 RNA 浓度及纯度，1%琼脂糖凝胶电泳检测 RNA 完整性及基因组污染情况，使用 SPARKscript II RT Plus Kit (with gDNA Eraser)反转录试剂盒去除基因组 DNA 并反转录成 cDNA。使用 2× SYBR Green qPCR Mix (with ROX)进行实时荧光定量 PCR (LightCycler® 480Ⅱ)分析。以核糖体蛋白 L17 (*rpl17*)为内参，按照$2^{-\Delta\Delta Ct}$计算目的基因相对表达量，引物序列见表 2。

1.6 数据统计

所有数据采用 SPSS 18.0 进行单因素方差分析(one-way ANOVA)，用 Duncan's 检验进行多重比较，$P<0.05$ 认为差异显著，$P>0.05$ 认为差异不显著。统计数据以平均值±标准误(Mean±SE)表示。

2 结果

2.1 精氨酸对 SBMIE-许氏平鲉生长性能和形体指标的影响

由表 3 可见，Arg 对 SBMIE-许氏平鲉生长性能

表 2 许氏平鲉肠道相关基因引物序列

Tab.2 Sequence of primers for gut related genes of *S. schlegelii*

基因名称 Gene names	正向引物 Forward primer (5′~3′)	反向引物 Reverse primer (5′~3′)
rpl17	AGGCGACGCACCTACCG	CCTCTGGTTTGGGGACGA
occludin	ACACCACAGGAGGAGAAACG	CTCTAAGGTCGGCATCAAATT
clnd15	TGCCGAACCGTTACTGGA	CGAATCCGTCGCACAAGA
zo-1	AACGCCGCAACAAAAGA	TGGCGGGGAAAGGATT
il-1β	TGGTTTCCCACGACTTCAC	TTTCGGTCACCAGGCTCT
IL-8	CTTATGGGACCCTGTTTGCT	TTCTTTAATCCACCCCTCGT
il-12b	CTCTGGCATCCTTATCAGTTCA	GTCTTGGTTGCTGGCGTAG
il-15	CGCCTACAATACAACTAAAGAGC	AGATGACGGAGCATACAGCA
tlr8	ACGTGATCGTGCTGCTGA	CCAAAACCAAGGCTCTGC

及形体指标具有显著影响。D2 和 D3 组许氏平鲉的末体重和增重率显著高于 D0 组(P<0.05)，D1 组与其他各组差异均不显著(P>0.05)；D1、D2 和 D3 组肝体比和脏体比均显著低于 D0 组(P<0.05)；D2 组肥满度显著高于其他各组(P<0.05)，D1 和 D3 组显著高于 D0 组(P<0.05)；各组间存活率差异不显著(P>0.05)。

2.2　精氨酸对 SBMIE-许氏平鲉血清精氨酸代谢相关酶活性及代谢产物的影响

由表 4 可见，添加 Arg 的各组许氏平鲉血清 DAO 活性、NO 含量及 iNOS 活性均显著低于 D0 组(P<0.05)，各处理组间无显著性差异(P>0.05)；D2 和 D3 组血清 T-NOS 活性显著低于 D0 组(P<0.05)，D1 组与其他各组间无显著性差异(P>0.05)。

2.3　精氨酸对 SBMIE-许氏平鲉肠道组织结构的影响

由表 5 可见，各处理组间皱襞数目和肌层厚度无显著性差异(P>0.05)，添加 Arg 的各组，皱襞高度显著高于 D0 组(P<0.05)，以 D2 组最高，D3 组与 D1、D2 组无显著性差异(P>0.05)。由图 1 可见，D0 组肠道黏膜受到损伤，固有层增宽(图 1-1)，杯状细胞数量增加，细胞核向单层柱状上皮细胞顶端移位，排列不规则(图 1-5)，添加 Arg 的各组，肠道黏膜结构完整，固有层增宽(图 1-3)和杯状细胞增多现象明显改善(图 1-2~4)，单层柱状上皮细胞排列规整，且细胞核整齐排列于细胞中下部(图 1-6~8)。

2.4　精氨酸对 SBMIE-许氏平鲉肠道抗氧化能力的影响

由表 6 可见，各处理组肠道 T-AOC 显著高于 D0 组(P<0.05)，D2 组显著高于其他各组(P<0.05)，D1 和 D3 组与对照组差异不显著(P>0.05)；随着 Arg 添加量的升高，肠道 MDA 含量呈先降低后升高的趋势，D2 和 D3 组显著低于 D0 组(P<0.05)，D1 组与其他各组差异不显著(P>0.05)。

表 3　精氨酸对 SBMIE-许氏平鲉生长性能和形体指标的影响

Tab.3　Effects of arginine on growth performance and body indexes of SBMIE-*S. schlegelii*

项目 Items	组别 Groups			
	D0	D1	D2	D3
初体重 IBW/g	54.90±0.03	54.92±0.01	54.93±0.05	54.99±0.03
末体重 FBW/g	81.51±0.39^{a}	83.18±0.67^{ab}	84.94±0.41^{b}	83.97±0.16^{b}
增重率 WGR/%	48.43±0.81^{a}	51.47±1.25^{ab}	54.65±0.81^{b}	52.68±0.44^{b}
肝体比 HSI/%	4.08±0.06^{b}	3.45±0.11^{a}	3.30±0.10^{a}	3.40±0.04^{a}
脏体比 VSI/%	10.39±0.22^{b}	9.56±0.08^{a}	9.29±0.07^{a}	9.33±0.12^{a}
肥满度 CF	2.45±0.03^{a}	3.24±0.10^{b}	3.65±0.07^{c}	3.30±0.05^{b}
存活率 SR/%	98.33±0.83	98.33±0.83	98.33±1.67	98.33±1.67

注：表格中同行肩标相同小写字母或无字母表示差异不显著(P>0.05)，不同小写字母表示差异显著(P<0.05)，下同。

Note: In the same row, data with same small letter superscripts or no letter superscripts mean no significant differences (P>0.05). Different small letter superscripts mean significant differences (P<0.05). The same below.

表 4　精氨酸对 SBMIE-许氏平鲉血清精氨酸代谢相关酶活性及代谢产物的影响

Tab.4　Effects of arginine on metabolism-related enzymes and arginine metabolites of SBMIE-*S. schlegelii*

项目 Items	组别 Groups			
	D0	D1	D2	D3
二胺氧化酶 DAO/(U/L)	122.80±6.33^{b}	92.66±3.13^{a}	96.61±3.70^{a}	97.78±6.43^{a}
一氧化氮 NO/(umol/L)	27.02±1.59^{b}	18.39±0.91^{a}	19.09±2.35^{a}	18.56±2.80^{a}
总一氧化氮合成酶 T-NOS/(U/mL)	21.21±0.78^{b}	19.34±0.57^{ab}	18.86±0.70^{a}	17.41±0.31^{a}
诱导型一氧化氮合成酶 iNOS/(U/mL)	15.77±0.77^{b}	11.88±1.22^{a}	11.62±0.63^{a}	11.29±0.19^{a}

表 5　精氨酸对 SBMIE-许氏平鲉肠道组织结构的影响

Tab.5　Effects of arginine on intestinal structure of SBMIE-*S. schlegelii*

项目 Items	组别 Groups			
	D0	D1	D2	D3
皱襞数目 Duplicature number	25.67±0.33	26.33±0.67	27.33±1.20	26.33±1.20
皱襞高度 Duplicature height/μm	295.22±13.15^{a}	414.05±8.04^{b}	505.72±26.57^{c}	468.47±23.77^{bc}
肌层厚度 Muscle thickness/μm	97.32±5.14	97.87±3.01	93.42±8.31	87.68±5.47

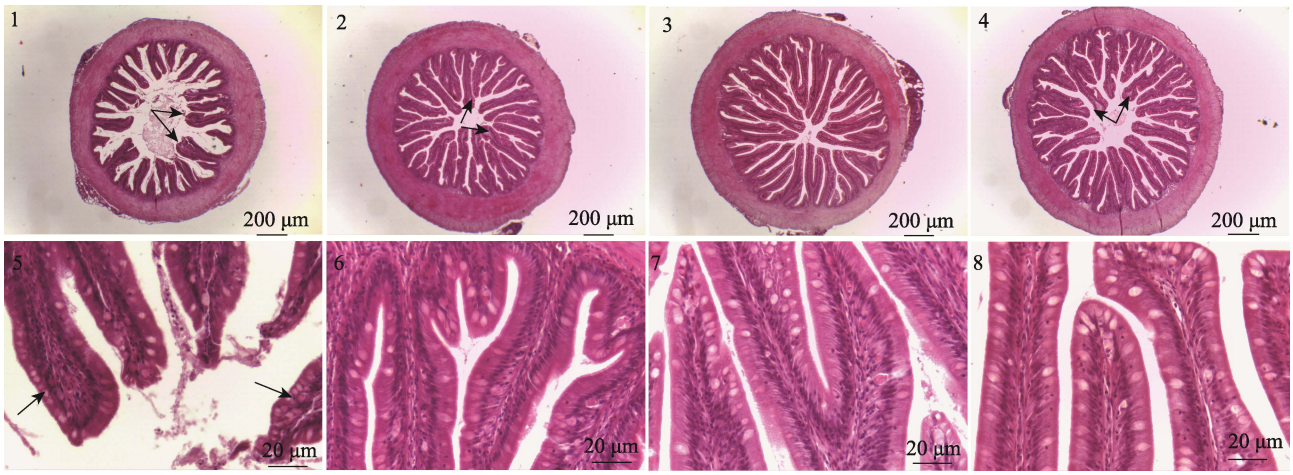

图 1 SBMIE-许氏平鲉肠道组织结构

Fig.1 Intestinal structure of SBMIE-*S. schlegelii*

图中 1~4 分别代表 40×下 D0、D1、D2、D3 组实验鱼肠道整体结构；5~8 分别代表 400×下 D0、D1、D2、D3 组肠道皱襞结构；1、2、4 中箭头所示固有层增宽，5 中箭头所示杯状细胞数量增加。

1~4 represent the overall structure of intestinal tract under 40× in group D0, D1, D2, and D3, respectively; 5~8 represent the intestinal fold structure under 400× in group D0, D1, D2 and D3, respectively. The arrows in figure 1, 2 and 4 indicated lamina propria widening, while the arrows in figure 5 show an increase in goblet cells.

表 6 精氨酸对 SBMIE-许氏平鲉肠道抗氧化能力的影响

Tab.6 Effects of arginine on intestinal antioxidant capacity of SBMIE-*S. schlegelii*

项目 Items	组别 Groups			
	D0	D1	D2	D3
总抗氧化能力 T-AOC /(mmol/g prot)	42.65±0.93^{a}	46.44±0.51^{b}	50.56±1.17^{c}	45.75±0.53^{b}
丙二醛 MDA /(nmol/mg prot)	1.68±0.10^{b}	1.45±0.05^{ab}	1.15±0.10^{a}	1.32±0.11^{a}

2.5 精氨酸对 SBMIE-许氏平鲉肠道紧密连接蛋白基因表达量的影响

由图 2 可见，相较于 D0 组，添加 Arg 的各组 *occludin* mRNA 相对表达量显著上调(P<0.05)，D1 和 D3 组显著高于对照 D0 组(P<0.05)，D2 组与其他各组均无显著差异(P>0.05)；D2 组显著高于 D0 和 D1 组(P<0.05)，D1 和 D3 组 *clnd15* mRNA 相对表达量与 D0 组差异不显著(P>0.05)，D3 组与 D0 和 D2 组差异不显著(P>0.05)；D1 组 *zo-1* mRNA 相对表达量显著高于其他各组(P<0.05)，D2 和 D3 组与 D0 组差异不显著(P>0.05)。

2.6 精氨酸对 SBMIE-许氏平鲉肠道炎症因子和抗炎因子基因表达量的影响

由图 3 可见，添加 Arg 各组许氏平鲉肠道 *il-1β* mRNA 相对表达量下调，D1 和 D2 组显著低于 D0 组(P<0.05)，D3 组与其他各组间无显著差异(P>0.05)；

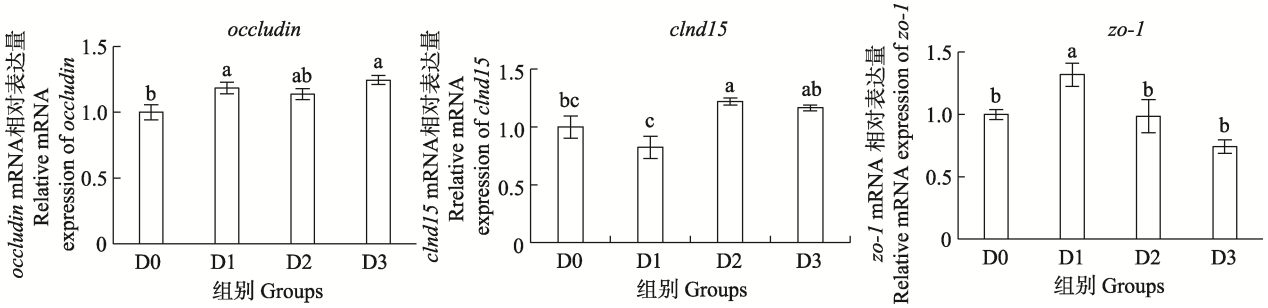

图 2 精氨酸对 SBMIE-许氏平鲉肠道紧密连接蛋白基因表达量的影响

Fig.2 Effects of arginine on intestinal tight junction protein gene expression of SBMIE-*S. schlegelii*

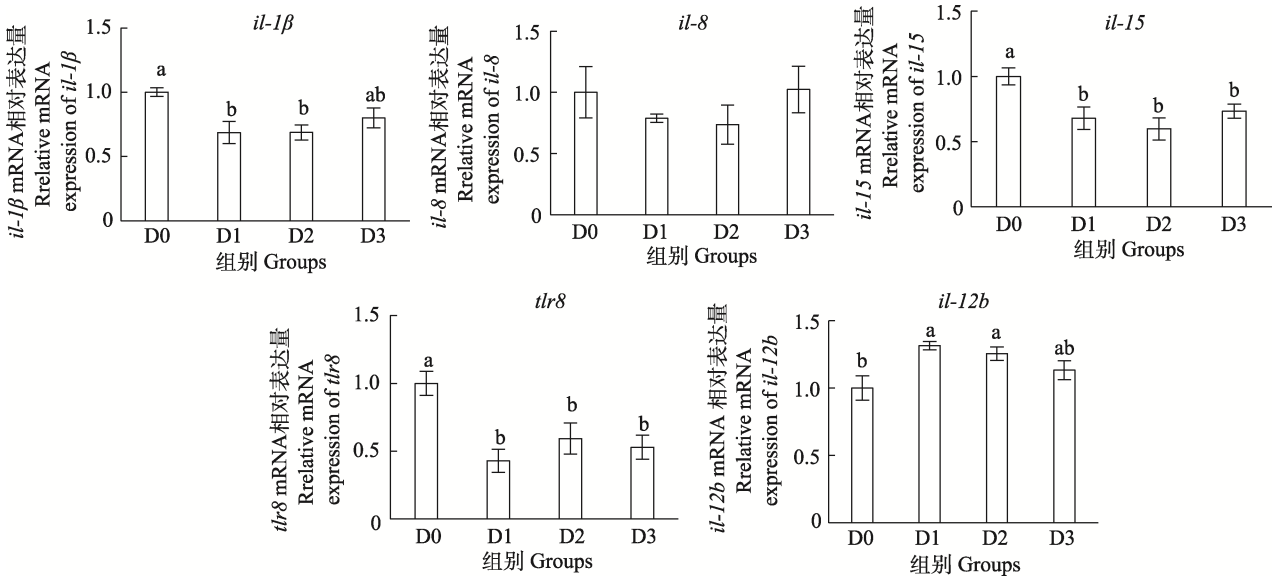

图 3　精氨酸对 SBMIE-许氏平鲉肠道炎症因子和抗炎因子基因表达量的影响

Fig.3　Effects of arginine on intestinal inflammatory and anti-inflammatory factors gene expression of SBMIE-*S. schlegelii*

Arg 对 *IL*-8 mRNA 相对表达量无显著影响(P>0.05)；各处理组 *il-15* 和 *tlr8* 的 mRNA 相对表达量显著低于 D0 组(P<0.05)，且各处理组间差异不显著(P>0.05)；D1 和 D2 组 *il-12b* mRNA 相对表达量显著高于 D0 组(P<0.05)，D3 组与其他各组差异不显著(P>0.05)。

3　讨论

对斜带石斑鱼(王彦飞, 2019)、花鲈(*Lateolabrax japonicus*)(王亚如, 2017)、黄金鲈(*Perca flavescens*)(吴莉芳等, 2017)等的研究均表明，长期摄食高豆粕饲料会影响生长性能。豆粕中含有大豆抗原蛋白、皂甙、植酸、非淀粉多糖等抗营养因子(Gu *et al*, 2016)，会降低饲料蛋白质的利用，增加内源蛋白质的分泌，最终导致粪氮增加(孙泽威等, 2005)。正常饮食情况下，健康许氏平鲉对 Arg 的最适需求量为 2.78% (沈钰博等, 2022)，本研究 D0 组饲料 Arg 水平为 2.799%，满足正常许氏平鲉的基本营养需求，但 SBMIE-许氏平鲉的肠道受到损伤，肠道功能的完整性直接影响鱼类生长性能，而在大菱鲆(Gu *et al*, 2017)、眼斑拟石首鱼(*Sciaenops ocellatus*)(Cheng *et al*, 2011)等的研究均已证明，Arg 通过改善肠道功能，进而影响实验鱼生长性能。本研究在基础配方 Arg 水平上额外添加 Arg，对许氏平鲉的增重率产生了显著的影响，2%的 Arg (D2)显著提高了 SBMIE-许氏平鲉的增重率，并且 Arg 的添加改善了肝体比、脏体比，提高了肥满度，推测 Arg 对 SBMIE-许氏平鲉的肠道产生了积极影响，从而提高了生长性能。

二胺氧化酶(DAO)是反映肠道机械屏障受损伤程度的细胞内酶，在小肠黏膜上皮细胞绒毛中含量高、活性强，若肠黏膜细胞受损、通透性增加，细胞内 DAO 会通过肠道屏障释放到细胞外进入血液(卓丽欣等, 2018)。本研究 Arg 显著降低了血清 DAO 活性，提示 D0 组肠道细胞膜通透性增加，而 Arg 显著降低了 DAO 的释放，保护了肠黏膜屏障的完整性。诱导型 NOS (iNOS)主要分布于巨噬细胞，一经产生就会催化生成大量 NO (Wang *et al*, 2009)。NO 参与上皮细胞迁移，形成新的上皮细胞，促进肠黏膜修复；在炎症反应中，致炎物可诱导局部 NO 合成与释放(孙红暖等, 2014)，过量 NO 会与体内氧自由基结合生成 $ONOO^-$和 NO^{2-}，导致肠黏膜损伤(Upperman *et al*, 2005)。大黄鱼(*Larimichthys crocea*)日粮添加 Arg 显著提高鱼血清 T-NOS 和肝脏 T-NOS、iNOS 活性，NO 显著升高(Zhou *et al*, 2012)；沈钰博等(2022)在许氏平鲉中也获得相同趋势，血清 NO 从对照组 4.82 μmol/L 提高到 19.26 μmol/L。本研究 Arg 添加组血清 T-NOS 和 iNOS 活性显著降低，血清 NO 从 D0 组 27.02 umol/L 降至 18.39~19.09 umol/L，与上述研究趋势相反，但 Arg 添加组的数值相符，原因可能是上述研究的实验鱼处于健康状态，适当添加 Arg 可提高 NOS 活性，从而增加 NO 的产生，而本研究 D0 组许氏平鲉肠道受到严重损伤，炎症反应激活了巨噬细胞 iNOS 的合成，增强了 NO 合成与释放，导致血清 NO 过量，而 Arg 可有效修复肠黏膜损伤，降低炎症反应，从而将

NO 的合成与释放控制在正常范围。

肠道结构的完整性与肠道吸收能力密切相关。对大西洋鳕鱼(*Gadus morhua*)(Refstie *et al*, 2006)、大菱鲆(Sun *et al*, 2022)、黄金鲈(吴莉芳等, 2017)等鱼的研究均表明，饲料中大量使用豆粕会导致肠道黏膜病理变化，且随着饲料豆粕比例升高及饲喂时间延长而越发严重。豆粕中含有较多的植物凝集素，与蛋白质和碳水化合物等有很强的结合能力，可作用于肠黏膜刷状缘，引起黏膜结构破坏及通透性增加(周红蕾等, 2006)。本研究 D0 组出现了皱襞高度降低、上皮细胞脱落、固有层增宽、杯状细胞数量增加等明显的 SBMIE 症状，添加 Arg 后肠炎症状明显改善，以 D2 组皱襞高度最高。在红鱼(*Sciaenops ocellatus*)(Cheng *et al*, 2011)和杂交条纹鲈鱼(*Morone chrysops*×*Morone saxatilis*)(Cheng *et al*, 2012)研究中也观察到类似结果：饲料添加 1% Arg (豆粕提供 40%饲料蛋白质)，鱼的皱襞高度、肠细胞高度和微绒毛高度均有所提高。以上均表明，Arg 在维护肠道黏膜结构完整性方面的积极作用。

T-AOC 高低体现机体清除活性氧自由基、保护细胞膜和细胞内核酸抵御自由基氧化损伤能力的高低(卓丽欣等, 2018)。MDA 是生物体内脂质氧化的终产物，具有细胞毒性，能间接反映机体是否存在氧化损伤。对大黄鱼(吴钊, 2016)、奥尼罗非鱼(*Oreochromis niloticus* ×*O. aureus*)(Lin *et al*, 2011)、黄金鲫(*Carassius auratus*)(王婧瑶等, 2022)等的研究均表明，豆粕过量替代鱼粉会降低鱼体抗氧化能力。本研究 D0 组肠道 T-AOC 最低而 MDA 含量最高，与上述结果一致。可能是 D0 组许氏平鲉长期大量摄入豆粕造成了肠道黏膜损伤，机体过量自由基得不到及时清除，产生大量脂质过氧化产物 MDA (李学丽等, 2017)。而添加 Arg 各组肠道 T-AOC 显著升高，MDA 显著降低，表明 Arg 可以改善由高剂量豆粕引起的机体氧化损伤，在草鱼(陈娇娇, 2017)、黄颡鱼(*Pelteobagrus fulvidraco*)(陈启明, 2016)上也得到了类似结论。Tan 等(2010)研究表明，Arg 能刺激细胞增殖并预防内毒素引起的肠细胞死亡，可能是 Arg 修复了受损的肠道黏膜，保护肠道细胞免受损伤，从而恢复了机体抗氧化能力。

肠道紧密连接通透性决定肠道黏膜屏障的功能，跨膜蛋白[如闭锁蛋白(occludin)和闭合蛋白(claudin，CLND)]以及胞质蛋白(如 zo-1)构成紧密连接的复杂蛋白质结构，zo-1 通过氨基端的 PDZ 结构域和 occludin、claudin 直接连接，在紧密连接的结构组成中发挥重要作用(孔瑶瑶等, 2020)。对珍珠龙胆石斑鱼 (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂)的研究表明，紧密连接相关基因 *occludin*、*clnd15a*、*zo-1* 在前、中、后肠表达量都较高，提示三者在维护肠道黏膜屏障功能上的关键作用(陈东鸿等, 2021)。*zo-1* 表达量在相当程度上受饲料营养素的影响和调节(孔瑶瑶等, 2020)，在 Arg (Chen *et al*, 2018)、谷氨酸(Jiang *et al*, 2017)、缬氨酸(Luo *et al*, 2014)上均得到证实。对大菱鲆的研究表明，40%豆粕显著降低了大菱鲆肠道紧密连接蛋白相关基因表达量而引发肠道炎症(Chen *et al*, 2018)。Arg 作为功能性氨基酸，可能主要通过改善肠道黏膜屏障功能来减轻肠道疾病，对珍珠龙胆石斑鱼(陈东鸿等, 2021)和草鱼(陈娇娇, 2017)的研究表明，Arg 可能通过增加 *occludin* 和 *clnd15*a 基因表达水平，加强与 ZOs 对紧密连接“锁扣”结构的“闭合”调控，从而保障肠黏膜屏障的功能。建鲤幼鱼饲料中 Arg 缺乏导致肠道紧密连接蛋白表达异常(Wang *et al*, 2016)。本研究 D0 组 30%的豆粕添加量肠道 *occludin*、*clnd15*、*zo-1* 三种紧密连接蛋白基因的相对表达量均受到影响，Arg 的添加显著上调了 3 个基因的相对表达量，与上述研究结果一致，表明 Arg 在修复肠黏膜屏障方面具有一定作用。

鱼类肠黏膜免疫状态与炎症反应密切相关，肠黏膜受损会引起通透性增加，细菌、毒素等会激活肠黏膜淋巴组织，引发免疫反应并释放大量促炎因子，影响肠上皮细胞对营养物质的吸收和对离子的转运(王亚如, 2017)。SBMIE 通常与白细胞介素(*il*)、*TLR*s 等促炎细胞因子基因的高表达有关(Zhao *et al*, 2019)，il-1β、IL-8、il-12b、il-15 和 tlr8 是肠道免疫的重要调节因子，在黏膜损伤或感染反应中起着关键作用(Wang *et al*, 2017)。il-1β 主要由活化的巨噬细胞和单核细胞产生，对肠上皮通透性有刺激作用(Planchon *et al*, 1994)。TLRs 能够识别肠道病原体相关分子，转化为参与肠道炎症控制的信号，使炎症处于控制之下(Zhao *et al*, 2019)。大菱鲆(Zhao *et al*, 2019)和斜带石斑鱼(Wang *et al*, 2017)摄食高剂量豆粕，肠道 *il-1β* 和 *TLR*s 相关分子基因表达上调，肠黏膜受损，而饲料中添加 1%Arg 显著抑制了大菱鲆肠道促炎因子的表达，提高了抗炎因子在后肠的表达(Chen *et al*, 2018)；在培养基中添加 Arg，显著降低了脂多糖诱导处理的细胞 *TLR4* 的表达(Tan *et al*, 2010)。本研究 Arg 添加组肠道炎症程度较 D0 组显著降低，与 *il-1β*、*IL*-8、*il-15* 和 *tlr8* 表达水平呈正相关，与 *il-12b* 表达水平呈负相关，提示 Arg 可通过降低促炎因子水平和提高抗炎因子水平缓解肠道炎症。另有研究表明，*il-1β* 等促炎因子能够抑制紧密连接蛋白基因的表达(石丹等, 2015)，推测 Arg 通过抑制促炎因子的表达，

从而促进细胞间紧密连接蛋白合成，增强鱼类上皮细胞间物理屏障功能。

4　结论

本实验条件下，高豆粕饲料中添加 Arg 能显著提高 SBMIE-许氏平鲉的生长性能和抗氧化性能，改善 Arg 代谢和肠道组织结构，上调肠道紧密连接蛋白和抗炎因子相关基因的表达，下调炎症因子相关基因的表达。因此，Arg (以 2%最佳)对许氏平鲉豆粕型肠炎具有修复作用。

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(编辑　陈　辉)

The Repairing Effect of Arginine on Soybean Meal-Induced Enteritis of *Sebastes schlegelii*

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Abstract　The complete intestinal structure is important to ensure the rapid and h ealthy growth of fish. However, the feed composition, aquaculture water environment, intestinal microbial population, and other factors may affect the intestinal health of fish. Intestinal health problems caused by feed ingredients are mainly due to the antinutrient factors contained in raw materials. Antinutritional factors contained in high-level soybean meal can cause oxidative damage to the intestine, thus, inducing soybean meal-induced enteritis (SBMIE), which leads to a decreased appetite and the slow growth of fish. Alleviating the damage of soybean meal to the fish intestinal tract and improving intestinal health through nutrition are essential methods for ensuring the sustainable development of the feed industry, which has significant ecological and economic significance.

As a functional amino acid, arginine is a precursor to the synthesis of bioactive substances, such as urea, glutamic acid, creatine, proline, polyamine, and nitric oxide. Arginine modulates metabolic regulation, including growth, immunity, intestinal barrier, and endocrine regulation. It plays a vital role in the immune regulation, maintenance, and protection of the intestinal mucosal structure and function. It has been reported that arginine is beneficial for repairing intestinal mucosal injury in poultry and aquatic animals. In this study, the carnivorous marine economic fish *Sebastes schlegelii* (54.97±0.12) g were used to investigate the repair effect and mechanism of arginine on SBMIE. This study aimed to provide a scientific basis for the application of arginine for maintaining the intestinal health of fish and provide a reference for the application of plant protein to the compound feed of the carnivorous economic fish *S. schlegelii*.

The purpose of this study was to investigate the repairing effects of arginine on the growth performance, arginine metabolism, intestinal structure, antioxidant performance, relative expression levels of intestinal tight junction protein genes (*occludin, clnd15*, and *zo-1*), and inflammatory factor-related genes (*il-1β*, *il-8, il-15*, and *tlr8*) and anti-inflammatory factor-related gene (*il-12b*) of *S. schlegelii* with SBMIE. *S. schlegelii* were fed high-level soybean meal (40%) for 28 days to induce SBMIE. SBMIE-*S. schlegelii* weighing (54.97±0.12) g were used as the study animals. Four

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isonitrogen and isoenergetic experimental feeds were formulated. The basic formula was supplemented with 30% soybean meal, arginine 0 supplementation as the control group (D0), and 1%, 2%, and 3% arginine supplementation as the treatment groups, named D1, D2, and D3, respectively. Each diet group had three replicates, and each replicate consisted of 40 fish. The fish were randomly placed in 12 homemade cages (60 cm × 60 cm × 90 cm). The experiment lasted for 6 weeks. The experimental fish were fed twice a day (08:00 and 17:00), with the initial feeding amount being 1% of the body weight, and the feeding amount being adjusted according to the feeding situation. During the experiment, the bottom of the cages was cleaned, and the water was changed every day to maintain the water temperature at 18~22 ℃, the dissolved oxygen at > 6 mg/L, the pH at 7.6~8.2, the ammonia nitrogen content at < 0.05 mg/L, and the nitrite nitrogen content at < 0.05 mg/L. The light cycle was the natural cycle. The results showed that the weight gain rate of the fish in the D2 and D3 groups was significantly higher than that in D0 group (P<0.05). The hepatosomatic and viscerosomatic indexes of the fish in the arginine treatment groups were significantly lower than those in the D0 group, and the condition factor was significantly higher than that in the D0 group (P<0.05). There was no significant effect on the survival rate (P>0.05). Diamine oxidase (DAO) activity, NO content, and iNOS activity values in the serum of the treatment groups were significantly lower than those in the D0 group (P<0.05). The serum T-NOS activity in the D2 and D3 groups was significantly lower than that in the D0 group (P<0.05). The duplicature height in the treatment groups was significantly higher than that in the D0 group, while no significant difference was found in the duplicature number and muscle thickness (P>0.05). In group D0, the intestinal mucosa lamina propria widened, and the number of goblet cells increased, while in groups supplemented with arginine, the intestinal mucosa was intact, and the problems mentioned above improved significantly. Intestinal total antioxidant capacity (T-AOC) in arginine supplementation groups was significantly increased, and the highest value was found in group D2 (P<0.05). The malondialdehyde content in groups D2 and D3 was decreased significantly compared to that in the D0 group (P<0.05). The relative expression of *occludin* mRNA in each treatment group was significantly upregulated compared to that in the D0 group (P<0.05). The relative expression level of *clnd15* mRNA in the D2 group was significantly higher than that in the D0 and D1 groups (P<0.05). The relative expression level of *zo-1* mRNA in group D1 was significantly higher than that in the other groups (P<0.05). The relative expression levels of *IL-1β*, *IL-15*, and *TLR8* mRNA were downregulated in all treatment groups, while the relative expression of *IL-12b* mRNA was upregulated (P<0.05). No significant differences were found in *IL-8* mRNA relative expression (P>0.05). In conclusion, under the conditions of this experiment, the growth and antioxidant performances of *S. schlegelii* with SBMIE were significantly increased, arginine metabolism and the intestinal structure were improved significantly, and the relative expression of intestinal tight junction protein and anti-inflammatory factor-related genes was upregulated, while that of inflammatory factor-related genes was downregulated, with arginine supplementation in a high-level soybean meal diet. Arginine (2% best) was effective in repairing SBMIE of *S. schlegelii*. The results of this study provide a theoretical basis for the mechanism of repairing SBMIE with arginine.

Key words *Sebastes schlegelii*; Arginine; Soybean meal-induced enteritis; Tight junction protein; Inflammatory factor