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饲料精氨酸水平对斜带石斑鱼蛋白质沉积和相关免疫基因表达的影响

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摘要：为研究饲料中不同水平精氨酸对斜带石斑鱼蛋白质沉积和相关免疫基因表达的影响，实验配制7种等氮等脂的饲料，精氨酸浓度分别为2.13%、2.42%、2.71%、2.95%、3.20%、3.48%和3.74%。随机挑选健康的斜带石斑鱼[初始体质量(80.11±0.03) g]分成7组，每组设3个重复，每个重复25尾鱼，进行为期10周的养殖实验。结果显示，精氨酸浓度为2.71%组鱼体增重率和特定生长率显著高于2.13%和2.42%组，饲料系数显著低于2.13%组。2.71%组的蛋白质效率显著高于2.13%组和3.48%组，2.71%组的蛋白质沉积率与2.95%组无显著差异，显著大于其他组。以增重率为依据，经折线模型拟合得出，斜带石斑鱼对饲料中精氨酸的最适需求量为饲料的2.73%(饲料蛋白质的5.40%)。斜带石斑鱼血清胰岛素在3.20%组达到最大值，与3.48%组差异不显著，显著高于其他组。肌肉雷帕霉素靶蛋白(TOR) mRNA水平3.48%组显著高于2.13%、2.42%及2.71%组。2.42%组后肠$b^{0,+}AT$基因表达量最高，显著高于其他各组。2.95%和3.20%组肾脏$b^{0,+}AT$基因表达量差异不显著，显著高于其他组。研究表明，适宜水平的饲料精氨酸可以刺激斜带石斑鱼胰岛素生长因子-Ⅰ(IGF-Ⅰ)的分泌，进而促进蛋白质的合成；提高鱼体肠道、肾脏及肝脏相关免疫基因的表达，提高机体免疫力，促进鱼体生长。

关键词：斜带石斑鱼；精氨酸；蛋白质沉积；免疫基因表达

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精氨酸作为鱼类生长的必需氨基酸，参与机体蛋白质的合成[1]，改善卵形鲳鲹(*Trachinotus ovatus*)[初始体质量(18.81±0.18) g] [2]、黑鲷(*Sparus macrocephalus*)[初始体质量(10.51±0.15) g] [3]、真赤鲷(*Pagrus major*)[初始体质量(13.3±0.2) g] [4]以及大西洋鲑(*Salmo salar*)(初始体质量110 g左右)[5]和吉富罗非鱼(GIFT *Oreochromis niloticus*)[初始体质量(81.52±2.00) g] [6]的增重率、特定生长率及蛋白质沉积率。精氨酸可以通过提高鱼肝脏中生长素(GH)和胰岛素生长因子-Ⅰ(IGF-Ⅰ)的转录水平[7-10]及血清中GH和IGF-Ⅰ的含量[11-12]，显著改善鱼体生长。IGF-Ⅰ与其受体(IGF-IR)特异结合，促使磷脂酰肌醇三激酶磷酸化，激活异育银鲫(*Carassis auratus gibelio*)相关组织雷帕霉素靶蛋白(TOR)进而通过调节核糖体蛋白S6激酶(S6K1)和翻译起始因子(4E-BPs)调节机体蛋白质合成，从而促进其生长[11, 13-14]。

鱼体免疫性能的强弱与其生长密切相关。饲料中精氨酸缺乏会降低舌齿鲈(*Dicentrarchus labrax*)[15]和银大麻哈鱼(*Oncorhynchus kisutch*)[16]的

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成活率。适宜水平精氨酸，可以改善斑点叉尾鮰(*Ictalurus punctatus*)抗病力[17]及其原代头肾细胞中巨噬细胞的数量和杀伤力[18]。精氨酸可以通过调控TOR、4*E-BPs*及*Nrf* 2 mRNA的表达来提高建鲤(*Cyprinus carpio* var. *Jian*)[19]和草鱼(*Ctenopharyngodon idella*)[20]的抗病能力，通过促进真赤鲷[4]、黄颡鱼(*Pelteobagrus fulvidraco*)[21]和尼罗罗非鱼(*Oreochromis niloticus*)[22]鱼体的一氧化氮含量来提高其免疫力。

本课题组前期研究表明，饲料精氨酸水平为3.06%时，可使斜带石斑鱼(*Epinephelus coioides*) [初始体质量为(7.52±0.02) g]具备良好的免疫力并获得最优的生长性能[23]。然而，随着鱼体逐渐生长，其饲料精氨酸的需求量对机体生长和抗病能力的响应是否与幼鱼一致呢？本实验选取斜带石斑鱼[初始体质量为(80.11±0.03) g]对其生长、蛋白质沉积及免疫指标作进一步研究和探讨。

1 材料与方法

1.1 实验设计与饲料

配制7种等氮等脂的实验饲料(表1)，分别添加*L*-精氨酸(99%, 上海三杰生物科技有限公司)，使饲料精氨酸含量分别达到2.13%、2.42%、2.71%、2.95%、3.20%、3.48%和3.74%。饲料原料粉碎过60目筛，根据配方比例逐级添加，混合

表 1 实验饲料配方

Tab. 1 Formulation and proximate analysis of trial diets

%

	精氨酸水平 arginine levels						
	2.13%	2.42%	2.71%	2.95%	3.20%	3.48%	3.74%
原料 ingredient							
白鱼粉 white fish meal	28.00	28.00	28.00	28.00	28.00	28.00	28.00
去皮豆粕 dehulled soybean meal	18.00	18.00	18.00	18.00	18.00	18.00	18.00
面粉 wheat flour	20.00	20.00	20.00	20.00	20.00	20.00	20.00
玉米蛋白粉 corn gluten meal	10.00	10.00	10.00	10.00	10.00	10.00	10.00
必需氨基酸 essential amino acids[1]	5.46	5.46	5.46	5.46	5.46	5.46	5.46
非必需氨基酸 nonessential amino acids[2]	4.51	4.21	3.91	3.61	3.31	3.01	2.71
精氨酸 arginine	0.00	0.30	0.60	0.90	1.20	1.50	1.80
其他 others[3]	14.03	14.03	14.03	14.03	14.03	14.03	14.03
合计 total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
主要营养成分(干物质) main nutrients							
精氨酸 arginine	2.13	2.42	2.71	2.95	3.20	3.48	3.74
粗蛋白 crude protein	50.48	50.31	50.19	50.44	50.49	50.50	50.40
粗脂肪 crude lipid	10.34	10.33	10.67	10.33	10.43	10.20	10.65
粗灰分 crude ash	10.60	11.51	11.24	11.79	11.32	11.61	11.24

注：1. 必需氨基酸组成(每100 g饲料)有赖氨酸 1.659 g、组氨酸 0.085 g、亮氨酸 0.627 g、异亮氨酸 0.477 g、蛋氨酸 0.441 g、苯丙氨酸 0.243 g、苏氨酸 1.308 g、缬氨酸 0.704 g；2. 非必需氨基酸组成(每100 g饲料)中甘氨酸∶天冬氨酸=1∶1；3. 其他(%)含有鱼油 3、豆油 3、大豆磷脂 1.5、磷酸二氢钙 1.60、氯化胆碱 0.50、诱食剂 0.10、维生素C 0.05、乙氧基喹啉 0.03、维生素预混料 0.20、矿物质预混料 0.20、微晶纤维素 3.85；维生素预混料的组成(每千克饲料)有硫胺素 25 mg、核黄素 45 mg、泛酸 60 mg、烟酸 200 mg、吡哆醇 20 mg、生物素 1.20 mg、维生素B_{12} 0.1 mg、肌醇 800 mg、叶酸 20 mg、维生素A 32 mg、维生素E 120 mg、维生素D_3 5 mg、维生素K_3 10 mg；矿物质预混料的组成(每千克饲料)含有氟化钠 2 mg、碘化钾 0.8 mg、氯化钴(1%) 50 mg、硫酸铜 10 mg、硫酸亚铁 80 mg、硫酸锌 50 mg、硫酸锰 60 mg、硫酸镁 1 200 mg、氯化钠 100 mg、沸石粉 1 447.2 mg

Notes: 1. essential amino acids (per 100 g diet) contain *L*-lysine 1.659 g, *L*-histidine 0.085 g, *L*-leucine 0.627 g, *L*-isoleucine 0.477 g, *L*-methionine 0.441 g, *L*-phenylalanine 0.243 g, *L*-threonine 1.308 g, *L*-valine 0.704 g; 2. nonessential amino acids (per 100 g diet) that glycine∶*L*-aspartic acid=1∶1; 3. others (%) contain fish oil 3, soybean oil 3, phospholipid 1.5, calcium monophosphate 1.60, choline chloride 0.50, phagostimulant 0.10, vitamin C 0.05, ethoxy quinoline 0.03, vitamin premix 0.20, mineral premix 0.20, microcrystalline cellulose 3.85; vitamin premix (per kg diet): vitamin B_1 25 mg, vitamin B_2 45 mg, vitamin B_3 60 mg, vitamin B_5 200 mg, vitamin B_6 20 mg, vitamin B_7 1.20 mg, vitamin B_{12} 0.1 mg, inositol 800 mg, folic acid 20 mg, vitamin A 32 mg, vitamin E 120 mg, vitamin D_3 5 mg, vitamin K_3 10 mg; mineral premix (per kg diet): sodium fluoride 2 mg, potassium iodide 0.8 mg, cobalt chloride (1%) 50 mg, cupric sulphate 10 mg, ferrous sulphate 80 mg, zinc sulphate 50 mg, manganese sulphate 60 mg, magnesium sulfate 1 200 mg, sodium chloride 100 mg, zeolite powder 1 447.2 mg

均匀后，压制成直径为3 mm的颗粒饲料，室温风干，分装放于−20 °C冰箱保存。

1.2 实验用鱼与饲养管理

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进行。斜带石斑鱼购于湛江东海岛浩利斜带石斑鱼苗种场，购回后驯化2周，挑选活力强、规格均匀的石斑鱼[初始体质量(80.11±0.03) g]525尾，随机分为7组，每组3个重复，静水养殖于1 m^3的玻璃钢桶，每天表观饱食投喂2次(08: 00和16: 00)，水温26 °C~30 °C，pH 7.7~8.0，24 h持续供氧，保持溶解氧≥6 mg/L，养殖10周。

1.3 样品采集与测定

样品采集　禁饲24 h后养殖实验结束，对每个桶实验鱼进行计数和称重。随机取3尾鱼进行尾静脉取血，4 °C 4 000 r/min 离心10 min，取上清液，保存于−80 °C冰箱，备测血清激素含量。用75%乙醇对取样台进行消毒，取样人员佩戴口罩和手套，并消毒，用灭过菌的剪刀镊子取肌肉、肝脏、肾脏及肠道置于装有RNAlater (Ambion Austin, TX, USA)的2 mL离心管中，于−80 °C冰箱保存，备测免疫基因的表达。

血清激素水平的测定　GH、IGF-Ⅰ和胰岛素(INS)含量的检测委托北京北方生物技术研究所(采用放射免疫分析法，以豚鼠抗猪胰岛素抗血清为抗体，^{125}I-标记胰岛素作为示踪剂)。

生长指标的计算公式　成活率(survival rate, SR, %)=N_t/N_0×100%；

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

特定生长率(special growth ratio, SGR, %/d)=$(\ln W_t-\ln W_0)/D$×100%；

饲料系数(feed coefficient ratio, FCR)=$W_f/(W_t-W_0)$；

蛋白质效率(protein efficiency ratio, PER, %)=$(W_t-W_0)/(W_f \times W_P)$×100%；

蛋白质沉积率(protein deposition rate, PDR, %)=$(W_t \times CP_t-W_0 \times CP_0)/(W_f \times W_P)$×100%；

式中，N_t为终末尾数，N_0为初始尾数，W_t为终末体质量(g)，W_0为初始体质量(g)，D为实验天数(d)，W_f为摄入饲料量(g)，W_P为饲料中的粗蛋白含量(%)，CP_t为终末鱼体的粗蛋白含量(%)，CP_0为初始鱼体的粗蛋白含量(%)。

1.4 实时荧光定量检测

采用实时荧光定量PCR法测定基因mRNA表达水平。用TRIzol提取组织总RNA，并检测其完整性(琼脂糖凝胶)和浓度(NanoDrop 2000，Thermo Fisher Scientific, Waltham, MA, USA)，进行反转录(PrimeScript™ RT reagent Kit with gDNA Eraser，TaKaRa)。实时荧光定量总体系为20 µL(TaKaRa SYBR Premix Ex *Taq*™ II Kit)。以*β-actin*为内参，采用$2^{-\Delta\Delta C_T}$法计算$b^{0,+}$氨基酸转运载体($b^{0,+}AT$)、Toll样受体22 (*TLR*22)、主要组织相容性复合体-Ⅱ (*MHC*-Ⅱ)、抗菌肽(*Hepcidin*)和*TOR*基因的相对表达量，相关引物序列见表2。

表 2　实时荧光定量特异性引物

Tab. 2　Primer sequence for real-time fluorescence quantitative

基因 genes	序列 (5′-3′) sequence	产物长度/bp product length	退火温度/°C annealing temperature	基因序列 GenBank ID
β-actin-QF	AAATCGCCGCACTGGTTG	195	60.0	AY510710.2
β-actin-QR	TCAGGATACCCCTCTTGCTCT		58.0	
$b^{0,+}$ *AT-QF*	CCTCCACTCCTTTCTACGCC	168	59.3	KR559237.1
$b^{0,+}$ *AT-QR*	CTTGTGCCAACATAACCATGC		59.0	
TOR-QF	CGACAGCGAGGTTGACAGC	154	59.6	JN850959.1
TOR-QR	CAGGGAGATGGAGCGGAAG		60.2	
TLR22-QF	TCGTGTTTATGGTGGCA	152	61.1	HQ456304.1
TLR22-QR	GTGGGTGTTGTAGGAGATG		61.1	
MHC-Ⅱ*-QF*	ATGAATGCCTTGCCTGAG	180	62.5	FJ447338.1
MHC-Ⅱ*-QR*	CTGCTGGGCCTTGTAGTT		63.5	
Hepcidin-QF	TGCTCGCCTTCATTTGC	190	63.2	GU391241.1
Hepcidin-QR	GTCGGGTAGCAGTAAGGAG		62.0	

1.5 数据统计与分析

采用SPSS17.0软件对实验数据进行单因素方差分析(One-Way ANOVA)，若差异显著(P<0.05)，再进行Duncan氏多重比较，结果均以平均值±标准差(means±SD)表示。

2 结果

2.1 饲料精氨酸水平对斜带石斑鱼生长指标和成活率的影响

饲料精氨酸浓度对实验鱼的SR未产生显著影响(P>0.05)。2.71%组斜带石斑鱼WGR和SGR达到最大值，显著高于精氨酸水平最低组和最高组(P<0.05)。2.13%组FCR与3.48%组相比无显著差异(P>0.05)，但是显著高于其他组(P<0.05)(表3)。

饲料精氨酸水平对斜带石斑鱼全鱼粗蛋白和粗灰分影响不显著(P>0.05)，3.20%组全鱼粗脂肪显著高于2.13%和2.42%组(P<0.05)。蛋白质沉积率随精氨酸水平升高呈先升高后降低趋势，其中在2.71%组达到最大值(表4)。

本实验条件下，以WGR为依据，经折线模型拟合得出，斜带石斑鱼对饲料中精氨酸最适需求量为2.73%，相当于饲料蛋白质的5.40%(y_1=137.81x−145.23，R^2=0.960 9；y_2=−10.45x+258.38，R^2=0.756 2)(图1)。

2.2 饲料精氨酸水平对斜带石斑鱼血清激素的影响

血清INS和GH浓度随着饲料精氨酸水平的升高呈先升高后降低趋势，且分别在3.20%和2.95%组达到最大值(表5)。对血清激素水平与生长指标进行相关性分析，得出IGF-Ⅰ与斜带石斑鱼WGR和SGR表现为显著性正相关(WGR=0.803，SGR=0.792, P<0.05)(表6)。

2.3 饲料精氨酸水平对斜带石斑鱼组织相关基因表达量的影响

后肠(a)和肝脏(c)的$b^{0,+}AT$基因mRNA表达量分别在2.42%和3.74%组达到最大值，且显著高于其余各组(P<0.05)。肾脏(b) 2.95%组与3.20%组的$b^{0,+}AT$基因mRNA表达量差异不显著(P>0.05)，显著高于其他组(P<0.05) (图2)。

肌肉中3.48%组鱼体的*TOR*基因mRNA表达量最高，显著高于2.13%、2.42%和2.71%组(P<

表 3 饲料精氨酸水平对斜带石斑鱼生长和饲料利用的影响

Tab. 3 Effect of dietary arginine level on growth performance and feed utilization of *E. coioides*

项目 items	精氨酸水平/% arginine level						
	2.13	2.42	2.71	2.95	3.20	3.48	3.74
成活率/% SR	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
增重率/% WGR	152.96±8.18^a	178.97±6.57^b	232.89±6.50^d	225.18±5.68^{cd}	222.26±4.12^c	223.55±1.79^{cd}	219.97±3.32^c
特定生长率/(%/d) SGR	1.65±0.06^a	1.83±0.04^b	2.15±0.04^d	2.11±0.03^{cd}	2.09±0.02^{cd}	2.09±0.01^{cd}	2.08±0.02^c
饲料系数 FCR	1.19±0.04^b	1.09±0.04^a	1.08±0.02^a	1.08±0.01^a	1.10±0.02^a	1.14±0.04^{ab}	1.11±0.07^a

注：数值用平均值±标准差(n=3)表示，上标字母不同表示差异显著(P<0.05)；下同

Notes: all date were expressed as mean±SD (n=3), values in the same line having different superscript letters are significantly different (P<0.05); the same below

表 4 饲料精氨酸对斜带石斑鱼体组成成分和蛋白质沉积率的影响

Tab. 4 Effect of dietary arginine level on body composition and protein deposition rate of *E coioides*

项目 items	精氨酸水平/% arginine level						
	2.13	2.42	2.71	2.95	3.20	3.48	3.74
粗蛋白/% crude protein	58.07±0.52	58.37±0.56	58.43±0.25	58.48±1.09	57.34±0.80	58.53±0.57	57.88±0.54
粗脂肪/% crude lipid	24.01±1.12^a	24.18±1.25^a	25.55±1.87^{ab}	25.50±0.25^{ab}	26.93±1.62^b	25.38±1.28^{ab}	25.58±0.59^{ab}
粗灰分/% crude ash	16.09±0.28	16.40±0.38	16.68±0.15	16.47±0.48	16.25±0.58	16.06±0.07	16.19±0.52
蛋白质效率/% PER	1.66±0.05^a	1.82±0.06^{bc}	1.85±0.03^c	1.83±0.02^{bc}	1.80±0.03^{bc}	1.74±0.07^{ab}	1.78±0.10^{bc}
蛋白质沉积率/% PDR	29.08±0.82^a	31.76±0.85^b	33.90±0.31^c	32.39±1.46^{bc}	31.33±0.06^b	31.57±1.74^b	31.82±1.47^b

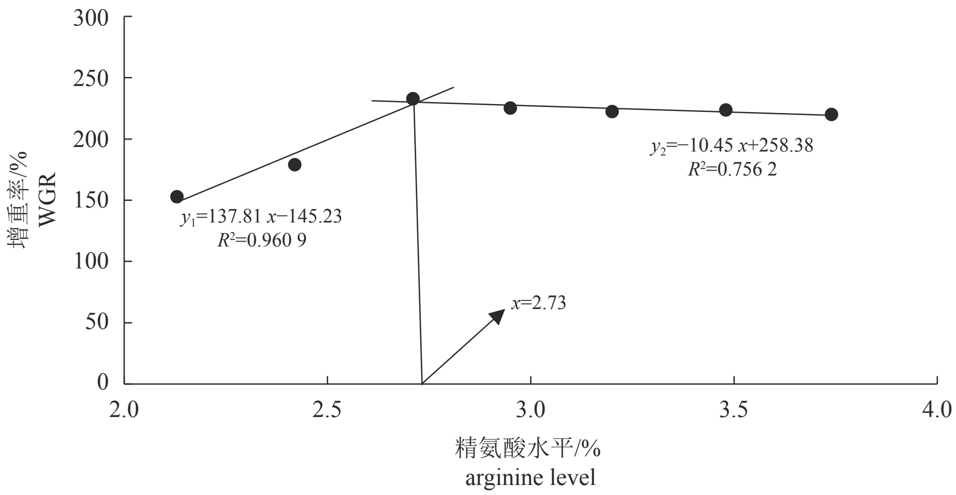


图 1　饲料精氨酸水平与斜带石斑鱼增重率的关系模式图

Fig. 1　Effect of dietary arginine level on weight growth rate of *E. coioides*

表 5　饲料精氨酸水平对斜带石斑鱼血清激素的影响

Tab. 5　Effect of dietary arginine level on serum hormone of *E. coioide*

项目 items	精氨酸水平/%　arginine level						
	2.13	2.42	2.71	2.95	3.20	3.48	3.74
胰岛素/(uIU/mL)　INS	5.86±0.43^a	6.02±0.40^a	5.93±0.42^a	7.56±1.80^a	10.17±1.99^b	7.80±1.94^{ab}	6.10±0.51^a
胰岛素样生长因子-Ⅰ/(ng/mL) IGF-Ⅰ	129.47±14.79^b	51.47±10.25^a	211.28±8.55^c	205.63±11.97^c	210.95±8.77^c	210.30±4.55^c	212.82±9.99^c
生长激素/(ng/mL)　GH	1.36±0.32^{ab}	1.68±0.61^{ab}	2.27±0.11^{bc}	3.52±0.38^d	2.74±0.88^{cd}	1.85±0.34^{abc}	1.20±0.08^a

表 6　斜带石斑鱼血清激素水平与生长指标相关性分析

Tab. 6　Correlation analysis between serum hormone level and growth of *E. coioides*

		项目　items	
		增重率/% WGR	特定生长率/(%/d) SGR
胰岛素 INS	相关系数 correlation factor	0.442	0.423
	*P*值	0.345	0.344
胰岛素样生长因子-Ⅰ IGF-Ⅰ	相关系数 correlation factor	0.803*	0.792*
	*P*值	0.03	0.034

注：*表示相关性为显著，显著水平为0.05，**表示相关性为极显著，显著水平为0.01；下同

Notes: * indicates a significant correlation, the significance level is 0.05; ** indicates an extremely significant correlation, the significance level is 0.01; the same below

0.05)(图3-a)。肠道中 2.13%和2.42%组的*TOR*基因mRNA表达量显著高于其余各组($P<0.05$)(图3-b)。

石斑鱼肠道中3.20%组*Hepcidin*基因mRNA表达量显著高于其他组($P<0.05$)；2.42%和2.71%组肠道*MHC*-Ⅱ基因和*TLR*22基因mRNA表达水平均显著高于其他组($P<0.05$)(图4-a)。肾脏中2.95%组*Hepcidin*基因表达量显著高于其余各组($P<0.05$)；*MHC*-Ⅱ基因表达水平随着精氨酸浓度的升高呈现降低的趋势，2.71%组显著高于(3.20%、3.48%和3.74%)组($P<0.05$)，与2.13%、2.42%和2.95%组差异不显著($P>0.05$)；随着精氨酸水平的升高，肾脏*TLR*22基因表达量呈先升高后降低的趋势，且在2.42%组达到最大值，显著大于2.13%、3.48%和3.74%组($P<0.05$)(图4-b)。肝脏(c) 2.13%、3.48%和3.74%组*Hepcidin*基因表达量显著低于其他组($P<0.05$)；肝脏*MHC*-Ⅱ表达量在2.42%组达到最大值且显著高于其他组($P<0.05$)；2.95%组和3.74%组*TLR*22基因表达量差异不显著($P>0.05$)，但显著高于其他组($P<0.05$)(图4-c)。

斜带石斑鱼组织和免疫基因相关分析性结果显示，饲料精氨酸水平对斜带石斑鱼肠道、肾脏和肝脏中*MHC*-Ⅱ表达量呈极显著负相关(r_i=−0.612，r_k=−0.860，r_l=−0.683，$P<0.01$)，*TLR*22基因表达量与肠道呈极显著负相关(r_i=−0.618，$P<0.01$)，与肝脏呈极显著正相关(r_l=0.580，$P<0.01$)，*Hepcidin*基因表达量与肝脏呈显著负相关(r_l=−0.528，$P<0.05$)(表7)。

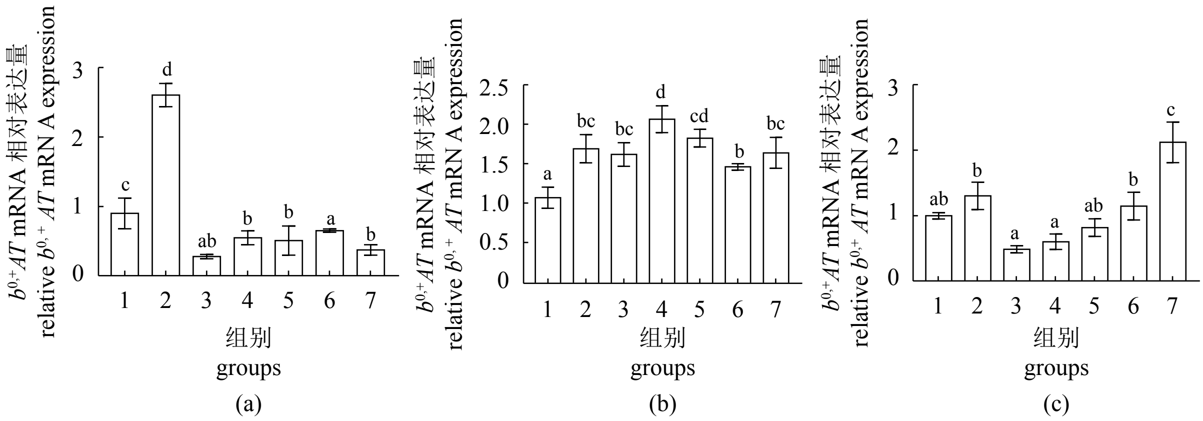


图 2　饲料精氨酸水平对斜带石斑鱼后肠(a)、肾脏(b)和肝脏(c)$b^{0,+}$ *AT*基因mRNA表达量的影响

1. 2.13%组，2. 2.42%组，3. 2.71%组，4. 2.95%组，5. 3.20%组，6. 3.48%组，7. 3.74%组；下同

Fig. 2　The $b^{0,+}$ *AT* mRNA expression in hindgut (a), kidney (b) and liver (c) of *E. coioides* fed diets containing different arginine levels

1. 2.13% group, 2. 2.42% group, 3. 2.71% group, 4. 2.95% group, 5. 3.20% group, 6. 3.48% group, 7. 3.74% group; the same below

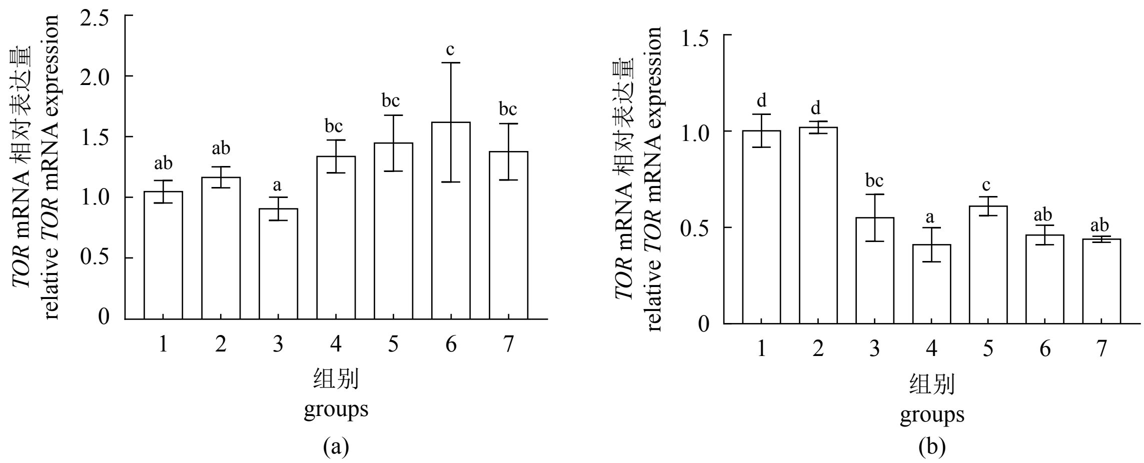


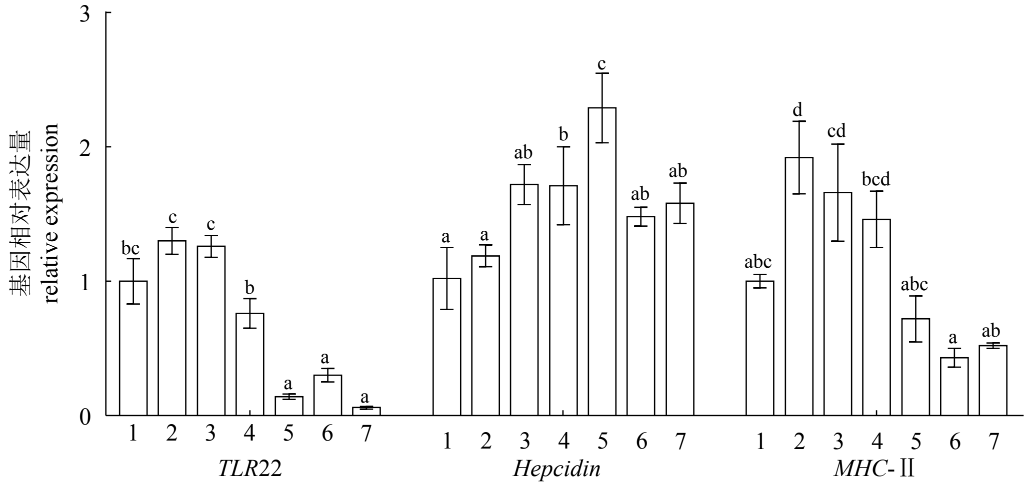
图 3　饲料精氨酸水平对斜带石斑鱼肌肉(a)和肠道(b)*TOR* mRNA表达量的影响

Fig. 3　The *TOR* mRNA expression in muscle (a) and intestine (b) of *E. coioides* fed diets containing different arginine levels

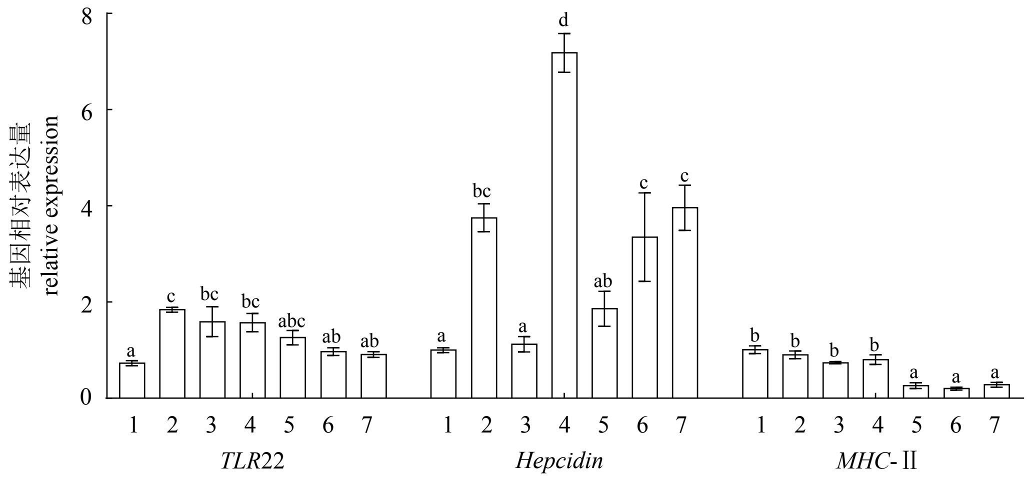
3　讨论

前期研究表明，小规格斜带石斑鱼(7.52±0.02) g获得最适生长的饲料精氨酸浓度为3.06%(饲料蛋白质含量的6.07%)[23]，而本实验中，当斜带石斑鱼鱼体规格较大时(80.11±0.03) g，饲料中适宜精氨酸含量为2.73%，表明随着鱼体的生长，斜带石斑鱼对精氨酸的需求量有所下降。随着饲料精氨酸水平的进一步升高，斜带石斑鱼的生长受到抑制，WGR和SGR显著下降，和大规格个体的吉富罗非鱼[24]和异育银鲫[11]对饲料精氨酸含量的响应方式一致，可能是摄入过多的精氨酸需要耗用机体能量进行脱氨，亦或者与赖氨酸在吸收时发生拮抗，降低对赖氨酸的吸收利用，进而抑制生长[25-27]。

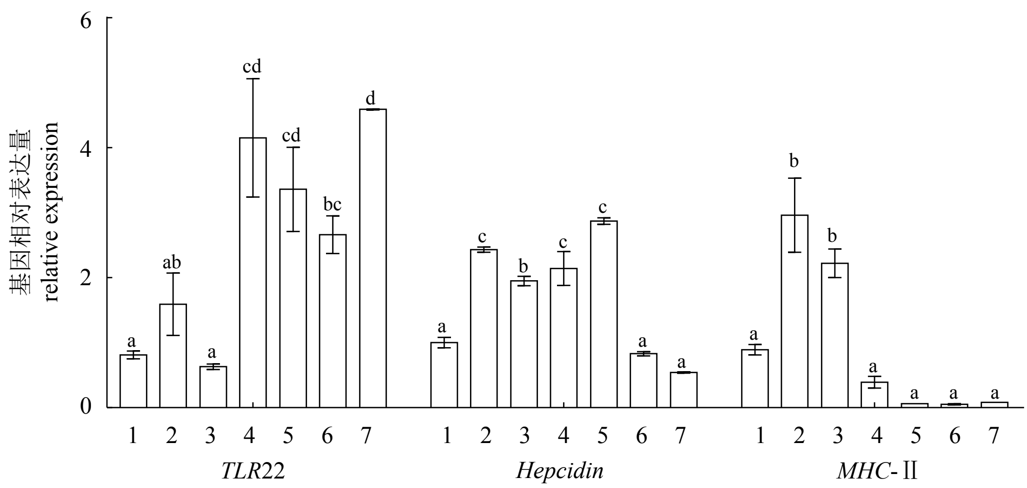
鱼类的生长受GH/ IGF-Ⅰ轴的调控[28-29]。GH是一种由垂体分泌的激素，通过结合GH受体刺激肝脏IGF-Ⅰ的合成与分泌，从而调控细胞增殖、分化及蛋白质合成[30]。团头鲂(*Megalobrama amblycephala*)[31]、大菱鲆(*Scophthalmus maximus*)[32]GH和IGF-Ⅰ基因表达的上调以及珍珠龙胆石斑鱼(*Epinephelus fuscoguttatus*♀× *E. lanceolatus*♂)[7]和斜带石斑鱼[12]血清GH和IGF-Ⅰ含量的增加均会促进鱼体生长。本实验中，2.71%组精氨酸水平下，血清IGF-Ⅰ浓度达到最大值，且有着最优的生长性能，且IGF-Ⅰ与其生长性能显著正相关，因此精氨酸对生长的影响可以通



(a)



(b)



(c)

图 4　饲料精氨酸水平对斜带石斑鱼中鱼肠道(a)、肾脏(b)和肝脏(c) *TLR*22 mRNA、*Hepcidin*和*MHC*-Ⅱ表达量的影响

Fig. 4　The *TLR*22, *Hepcidin* and *MHC*-Ⅱ mRNA relatiive expression in the intestine (a), kidney (b) and liver (c) of *E. coioides* fed diets containing different arginine levels

表 7 斜带石斑鱼组织与免疫基因相关性分析

Tab. 7 Correlation analysis between tissues and immune genes of *E. coioides*

r		项目 items		
		*TLR*22	*MHC*-Ⅱ	*Hepcidin*
肠道 intestine	相关系数 r_i correlation factor	−0.618**	−0.612**	−0.076
	P值	0.003	0.003	0.745
肾脏 kidney	相关系数 r_k correlation factor	−0.421	−0.860**	0.399
	P值	0.057	0	0.073
肝脏 liver	相关系数 r_l correlation factor	0.580**	−0.683**	−0.528*
	P值	0.006	0.002	0.014

过血清IGF-Ⅰ的变化来评价，提示精氨酸可以通过促进IGF-Ⅰ的分泌来促进鱼体生长。IGF-Ⅰ还是一种有效的促分泌激素，可影响GH、INS、胰高血糖素与催乳素的分泌[7]。血清GH水平的变化规律和石斑鱼SGR一致，表明精氨酸可通过促进GH的分泌促进斜带石斑鱼的生长。当饲料精氨酸水平超过2.95%，石斑鱼血清GH含量下降，可能是IGF-Ⅰ对GH负反馈的结果[33]。

IGF-Ⅰ是激活机体蛋白质合成信号通路(Akt-TOR)的上游信号分子之一[34-37]。机体蛋白质代谢处于动态平衡状态，当合成速率大于分解速率时会促进蛋白质沉积，表现为鱼体生长，而蛋白质的合成会受到翻译启动的限制，TOR可以通过下游S6K1和4E-BPs启动翻译并刺激蛋白质的合成[38]。本实验中，TOR mRNA的表达在石斑鱼肌肉中的变化趋势与血清IGF-Ⅰ变化趋势一致，提示精氨酸可能刺激IGF-Ⅰ的分泌，激活TOR通路促进机体蛋白的合成[13]。珍珠龙胆石斑鱼[39]肝脏IGF-Ⅰ和TOR mRNA的表达变化趋势与本实验结果一致。IGF-Ⅰ还可激活金头鲷(*Sparus aurata*)原代肌细胞*TOR*的表达[40]，从细胞水平印证了本实验的结果。肠道TOR mRNA水平随精氨酸水平升高呈逐渐下降趋势，可能是过多精氨酸通过降低肠道细胞TOR mRNA表达量，降低过多精氨酸带来的副作用，从而维持肠道细胞对精氨酸的利用合成蛋白质[41]。

氨基酸经由氨基酸转运载体进入细胞，同时氨基酸浓度也会影响转运载体的表达水平[42]。$b^{0,+}$系统属于Na^+非依赖性的碱性氨基酸转运载体[43]，主要在肾脏和肠道中表达。本实验中，适宜水平精氨酸会上调斜带石斑鱼后肠、肾脏与肝脏组织中$b^{0+}AT$ mRNA表达。然而随着精氨酸水平进一步升高，后肠和肾脏$b^{0+}AT$ mRNA表达量又下降，符合“自适应抑制理论”[44-45]，即当精氨酸水平较高时会抑制$b^{0+}AT$ mRNA的表达，来降低机体过量精氨酸的吸收带来的副作用。埃及伊蚊(*Aedes aegypti*)的研究发现，氨基酸转运载体基因的敲落会降低氨基酸诱导的*TOR*信号的表达[46]。本实验中，斜带石斑鱼肠道*TOR*和$b^{0+}AT$ mRNA表达变化趋势相似，得出饲料中适宜水平精氨酸会上调$b^{0+}AT$的表达，帮助精氨酸进入细胞，激活*TOR*的表达。

精氨酸除了可以促进生长外，还影响着机体免疫性能[47]。肠道和肾脏是机体中重要的吸收和免疫组织。*TLR*22是水生生物特有的*Toll*样受体，能识别病原体的保守分子模式(*PAMPs*)并激活它的表达[48-50]。*MHC*-Ⅱ能够识别外源性抗原表面的短肽，呈递给T细胞来消灭抗原，增强机体抗病菌感染力。精氨酸处理的小鼠会产生更多的脾髓样状树突细胞和浆细胞样状树突细胞，上调*MHC*-Ⅱ、*CD*86与*TLR*9 mRNA表达水平[51-52]，可以阻止肿瘤存在时树突状细胞MHC-Ⅱ水平的降低[53]。本研究显示适宜精氨酸水平有助于上调肠道和肾脏组织中*TLR*22基因表达，提高机体的免疫水平。随饲料精氨酸水平升高，石斑鱼肾脏、肠道和肝脏中*MHC*-Ⅱ mRNA表达量呈下降趋势，表明高水平的精氨酸可能加剧了机体炎症反应，从而抑制*MHC*-Ⅱ在肾脏、肠道和肝脏中的表达。斜带石斑鱼组织和免疫基因相关性分析的结果显示：*MHC*-Ⅱ对精氨酸的响应在后肠、肾脏和肝脏组织中显著负相关，*TLR*22对精氨酸的响应在肠道呈负相关，与肾脏呈正相关。即研究精氨酸对斜带石斑鱼肠道和肾脏这3个免疫基因时，选择*TLR*22和*MHC*-Ⅱ会比*Hepcidin*更有说服力。

在本实验条件下，斜带石斑鱼对饲料中精氨酸最适需求量为2.73%(相当于饲料蛋白质的5.40%)，适量精氨酸可以通过促进血清IGF-Ⅰ的分泌并激活*TOR*信号通路促进蛋白质沉积，同时提高机体免疫力，从而促进斜带石斑鱼的健康生长。

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Effects of dietary arginine levels on protein deposition and related immune gene expression in *Epinephelus coioides*

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Abstract: A 10-week feeding trial was conducted to investigate the effects of dietary arginine levels on the growth performance and immunity of *Epinephelus coioides*. Seven isonitrogenous and isolipid diets were formulated with arginine contents of 2.13%, 2.42%, 2.71%, 2.95%, 3.20%, 3.48% and 3.74% (dry matter) respectively. Randomly selected healthy groupers [initial weight (80.11±0.03) g] were divided into 7 groups, with 3 replicates in each group and 25 fish per replicate. It was found that the weight gain rate (WGR) and specific growth rate (SGR) of 2.71% group were significantly higher than 2.13% and 2.42% groups, and the feed coefficient (FCR) was significantly lower than that 2.13% group. The protein efficiency rate of the 2.71% group was significantly higher than those of 2.13% and 3.48% groups, and the protein deposition rate of the 2.71% group was not significantly different from that of 2.95% group, which was significantly higher than that of other groups. Broken-line model analysis of weight growth rate against dietary arginine levels indicated that the optimum requirement of arginine for maximum growth of subadult grouper was 2.73%, corresponding to 5.40% of dietary protein on a dry weight basis. There was no significant difference between 3.20% serum insulin (INS) and 3.48% groups, which were significantly higher than other groups. The level of *TOR* gene in muscle of 3.48% group was significantly higher than those of 2.13%, 2.42% and 2.71% groups. The expression of $b^{0,+}$ *AT* gene in the hindgut of 2.42% group was the highest, which was significantly higher than that of other groups. There was no significant difference in the expression of $b^{0,+}$ *AT* gene of kidney between 2.95% and 3.20% groups, which was significantly higher than that of other groups. In conclusion, the appropriate level of feed arginine can stimulate the secretion of IGF-I of *E. coioides* to promote the synthesis of protein and improve the expression of immune genes of the intestine, kidney and liver of fish to improve the body immunity, and ultimately promote the growth of fish.

Key words: *Epinephelus coioides*; arginine; protein deposition; immune gene expression

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