

低鱼粉饲料添加赖氨酸对异育银鲫“中科5号”幼鱼生长性能及代谢的影响

许文婕 王宇 巫丽云 董博 朱晓鸣 韩冬 刘昊昆 杨云霞 张志敏 解绶启 金俊琰

DIETARY LYSINE SUPPLEMENTATION ON GROWTH PERFORMANCES AND METABOLISM IN JUVENILE GIBEL CARP (*CARASSIUS GIBELIO* VAR. CAS V) FED LOW FISH MEAL DIETS

XU Wen-Jie, WANG Yu, WU Li-Yun, DONG Bo, ZHU Xiao-Ming, HAN Dong, LIU Hao-Kun, YANG Yun-Xia, ZHANG Zhi-Min, XIE Shou-Qi, JIN Jun-Yan

在线阅读 View online: <https://doi.org/10.7541/2024.2024.0246>

您可能感兴趣的其他文章

Articles you may be interested in

低蛋白饲料补充必需氨基酸对异育银鲫生长、氨基酸转运和mTOR信号通路相关基因的影响

LOW-PROTEIN DIETS SUPPLEMENTED WITH ESSENTIAL AMINO ACIDS ON GROWTH, AMINO ACID TRANSPORTERS AND GENE EXPRESSION OF THE MTOR SIGNALING PATHWAY IN GIBEL CARP (*CARASSIUS GIBELIO*)

水生生物学报. 2024, 48(1): 1–9 <https://doi.org/10.7541/2023.2023.0093>

饲料中添加椭圆栅藻对异育银鲫中科5号越冬后抗病力的影响

EFFECTS OF DIETARY *SCENEDESMUS OVALTERNUS* ON THE DISEASE RESISTANCE OF OVERWINTERING GIBEL CARP (*CARASSIUS GIBELIO* VAR. CAS V)

水生生物学报. 2022, 46(4): 457–465 <https://doi.org/10.7541/2021.2021.062>

饲料混合植物油替代鱼油对不同品种异育银鲫生长性能和脂肪代谢的影响

TOTAL REPLACEMENT OF FISH OIL BY BLENDED VEGETABLE OIL ON GROWTH PERFORMANCE AND LIPID METABOLISM IN DIFFERENT STRAINS OF GIBEL CARP (*CARASSIUS GIBELIO*)

水生生物学报. 2024, 48(4): 600–609 <https://doi.org/10.7541/2024.2022.0482>

饲料中添加高剂量有机硒和无机硒对异育银鲫生长性能、组织硒蓄积和血液生化指标的影响

HIGH DIETARY DOSES OF ORGANIC AND INORGANIC SELENIUM ON GROWTH PERFORMANCE, TISSUE SELENIUM ACCUMULATION AND BLOOD BIOCHEMICAL PARAMETERS OF GIBEL CARP (*CARASSIUS AURATUS GIBELIO* VAR. CAS V)

水生生物学报. 2023, 47(5): 702–712 <https://doi.org/10.7541/2023.2022.0208>



关注微信公众号，获得更多资讯信息

doi: 10.7541/2024.2024.0246

CSTR: 32229.14.SSSWXB.2024.0246

低鱼粉饲料添加赖氨酸对异育银鲫“中科5号”幼鱼生长性能及代谢的影响

许文婕^{1*} 王宇^{2,3*} 巫丽云² 董博^{2,3} 朱晓鸣² 韩冬² 刘昊昆²
杨云霞² 张志敏² 解绶启^{2,3} 金俊琰²

(1. 广东海洋大学食品科学与工程学院, 阳江 529500; 2. 中国科学院水生生物研究所淡水生态与生物技术国家重点实验室, 武汉 430072; 3. 中国科学院大学, 北京 100049)

摘要: 实验以初重(8.70±0.03) g的异育银鲫“中科5号”(Carassius gibelio var. CAS V)幼鱼为研究对象, 分别投喂低鱼粉饲料中添加0、2%、4%赖氨酸的饲料49d, 以探究低鱼粉饲料中添加不同水平赖氨酸对异育银鲫“中科5号”生长性能、鱼体生化组成、营养沉积、血浆代谢物及肌肉中赖氨酸感知和应答、肌肉生长相关基因表达的影响。实验结果显示, 饲料中添加不同水平的赖氨酸对异育银鲫“中科5号”的摄食率、特定生长率、饲料效率、蛋白沉积率、脂肪沉积率没有显著性影响($P>0.05$)。饲料中添加2%赖氨酸组, 全鱼蛋白质含量显著高于其他组($P<0.05$), 全鱼总氨基酸含量、总必需氨基酸含量显著高于其他两个处理组($P<0.05$), 其中赖氨酸、精氨酸、异亮氨酸、亮氨酸、苯丙氨酸、苏氨酸和缬氨酸等7种必需氨基酸含量显著高于其他各组($P<0.05$)。肌肉的氨基酸组成在各组间无显著性差异($P>0.05$)。血浆葡萄糖、甘油三酯和总胆固醇含量在各组间无显著性差异($P>0.05$)。血浆游离氨基酸组成没有受到饲料赖氨酸水平的显著影响($P>0.05$)。饲料中添加赖氨酸对肌肉中赖氨酸感知和应答相关关键基因表达无显著性影响($P>0.05$), 4%赖氨酸组肌肉myog的基因表达量高于2%赖氨酸组($P<0.05$)。结果表明, 低鱼粉饲料中添加2%赖氨酸可以在一定程度上提高异育银鲫“中科5号”全鱼必需氨基酸和蛋白质含量, 可为异育银鲫“中科5号”的配方设计提供支撑。

关键词: 赖氨酸; 生长; 氨基酸感知; 代谢; 异育银鲫“中科5号”

中图分类号: S965.1 **文献标识码:** A **文章编号:** 1000-3207(2025)03-032508-11



水产养殖在保障我国粮食安全和国民营养中发挥着重要作用。水产饲料中蛋白质是重要的营养成分, 占饲料成本的60%以上^[1]。水产养殖高度依赖鱼粉, 但鱼粉资源紧缺已经成为限制我国水产饲料行业发展的主要瓶颈。植物蛋白源替代鱼粉易造成赖氨酸等必需氨基酸的缺乏, 可能会导致鱼类摄食减少、生长缓慢、饲料转化率降低等问题。外源氨基酸添加可以缓解这一问题, 在低鱼粉饲料中补充赖氨酸可以促进花鲈(*Lateolabrax japonicus*)^[2]、鲤(*Oncorhynchus mykiss*)^[3]等鱼类的特定

生长率和饲料效率。

目前, 低鱼粉饲料中添加赖氨酸对鱼类影响的研究主要集中于生长性能和体成分, 对鱼类代谢的影响机制研究相对较少。必需氨基酸不仅是蛋白质的重要组成部分, 同时具备调控机体代谢的功能^[4]。有报道表明氨基酸特别是赖氨酸、精氨酸等碱性氨基酸是G蛋白耦联受体C家族6组A亚型(G protein coupled receptor family C, group 6, subtype A)的重要激动剂, GPRC6A被激活后诱导β-抑制蛋白(β-arrestins)的募集反应并与细胞内的G蛋白偶联, 最

收稿日期: 2024-06-18; 修订日期: 2024-07-16

基金项目: 国家大宗淡水鱼产业技术体系(CARS-45-09); 国家自然科学基金(32102811); 广东海洋大学科研启动经费项目资助[Supported by the Earmarked Fund for China Agriculture Research System (CARS-45-09); the National Natural Science Foundation of China (32102811); Program for Scientific Research Start-up Funds of Guangdong Ocean University]

作者简介: 许文婕(1990—), 女, 博士; 主要从事鱼类分子营养学研究。E-mail: xuwenjie@gdou.edu.cn 王宇(1999—), 男, 博士; 主要从事鱼类分子营养学研究。E-mail: wangyu001@ihb.ac.cn *共同第一作者

通信作者: 金俊琰(1983—), E-mail: jinjunyan@ihb.ac.cn

终参与调节胞外信号调节蛋白激酶(Extracellular regulated kinase, ERK)等信号转导途径^[5]。ERK通过抑制结节性硬化症蛋白复合体(Tuberous sclerosis complex, TSC), 进而通过脑组织中丰富表达的Ras同源类似物(Ras homolog enriched in brain, Rheb)鸟苷三磷酸酶(GTPase)激活mTOR通路, 最终调节机体摄食、营养代谢和吸收^[6-9]。mTOR信号通路是鱼类感知体内氨基酸浓度的主要信号通路, 位于蛋白质合成和降解过程中整个信号通路的中心, 通过调控蛋白质代谢等多种途径发挥重要的生理功能^[10, 11]。在大菱鲃(*Scophthalmus maximus* L.)^[12]、黄颡鱼(*Pelteobagrus fulvidraco*)^[13]、异育银鲫“中科3号”(*Carassis auratus gibelio* var. CAS III)^[14]等鱼类中的研究显示, 饲料中氨基酸不平衡或者某种氨基酸的缺乏会通过mTOR通路调控鱼体的代谢。mTOR信号通路的激活可以促进鱼类肌肉蛋白沉积及肌纤维的生长和发育^[15]。因此, 进一步探究鱼类对赖氨酸的感知和应答机制, 特别是肌肉组织对赖氨酸的应答机制, 可为提高鱼类对蛋白的利用提供科学依据。

异育银鲫(*Carassius gibelio*)是我国重要的淡水经济鱼类, 养殖年产量超280万吨^[16]。异育银鲫“中科5号”(*Carassius gibelio* var. CAS V), 2018年被国家原良种委员会审定水产养殖新品种, 具有生长快、可以更好的利用低鱼粉饲料等优点^[17]。虽然异育银鲫“中科5号”利用低鱼粉饲料能力较其他品种异育银鲫高, 但摄食低鱼粉饲料的生长性能仍然低于高鱼粉饲料。因此, 本研究以异育银鲫“中科5号”为研究对象, 探究低鱼粉饲料中添加不同水平赖氨酸对鱼体生长、体成分和肌肉生长代谢的影响, 以期为提高鱼类对低鱼粉饲料利用提供理论依据。

1 材料与方法

1.1 实验饲料

本实验以低鱼粉饲料(5%鱼粉)作为基础饲料, 根据异育银鲫幼鱼的赖氨酸需求, 设置低赖氨酸组(LL)、适宜赖氨酸组(ML)、高赖氨酸组(HL), 即分别添加0、2%、4%赖氨酸(纯度 $\geq 98\%$, 上海源叶生物科技有限公司, 中国上海), 饲料配方如表1所示。饲料原料中鱼粉购自美国海鲜公司, 小麦蛋白粉、豆粕和菜粕购自武汉高龙饲料有限公司。所有原料过40目筛后充分混匀, 加水调制后用制粒机(SLP-45, 上海渔业机械设备研究所, 中国)制粒, 于烘箱中70℃烘干后4℃保存。饲料配方如表1所示。

1.2 实验鱼和实验管理

实验所用异育银鲫“中科5号”来自中国科学院

水生生物研究所官桥渔场。养殖实验在长江故道网箱中进行(湖北省石首市老河长江四大家鱼原种场, 北纬 N29°49', 东经E112°28', 鱼池网箱尺寸为2 m×2 m×2 m, 水深约2.5 m)。在正式养殖实验开始前, 先将实验鱼饥饿24h, 随机挑选规格均匀、体质健康异育银鲫“中科5号”幼鱼[初重: (8.70±0.03) g]进行实验。实验共3个处理组, 每组3个平行, 每网

表1 饲料配方和营养组成(g/kg干重)

Tab. 1 Ingredient and proximate composition of the experimental diets (g/kg dry matter)

原料Ingredient	LL	ML	HL
白鱼粉White fish meal ¹	50	50	50
小麦蛋白粉Wheat gluten meal ²	200	175	150
豆粕Soybean meal ²	140	140	140
菜粕Rapeseed meal ²	140	140	140
面粉Wheat flour	250	250	250
赖氨酸 Lysine	0	20	40
混合油Oil mixture ³	80	80	80
维生素预混物Vitamin premix ⁴	3.9	3.9	3.9
矿物盐预混物Mineral premix ⁵	50	50	50
氯化胆碱Choline chloride	1.1	1.1	1.1
羧甲基纤维素钠Carboxymethyl cellulose	30	30	30
纤维素Cellulose	55	60	65
营养组成Proximate composition (g/kg)			
水分Moisture	66.3	45.7	54.1
粗蛋白Crude protein	351.7	354.0	351.0
粗脂肪Crude lipid	89.6	86.0	82.2
灰分Ash	62.5	63.2	66.2
赖氨酸Lysine	17.7	39.4	68.2

注: ¹鱼粉: 美国海鲜公司; ²小麦蛋白粉、豆粕、菜粕: 武汉高龙饲料有限公司; ³混合油: 豆油: 鱼油=1:1; ⁴维生素预混物(mg/kg 饲料): 维生素A, 1.65; 维生素D, 0.025; 维生素E, 50; 维生素K, 10; 维生素C, 100; 硫胺素, 20; 核黄素, 20; 维生素B6, 20; 维生素B12, 0.02; 叶酸, 5; 泛酸钙, 50; 肌醇, 100; 烟酸, 100; 生物素, 0.1; 纤维素, 645.2; ⁵矿物盐预混物(mg/kg 饲料): 氯化钠, 500; 七水合硫酸镁, 8155.6; 二水合磷酸二氢钠, 12500.0; 磷酸二氢钾, 16000.0; 二水合磷酸氢钙, 7650.6; 七水合硫酸亚铁, 2286.2; 五水合乳酸钙, 1750.0; 七水合硫酸锌, 178.0; 一水合硫酸锰, 61.4; 五水合硫酸铜, 15.5; 七水合硫酸钴, 0.5; 碘化钾, 1.5; 玉米淀粉, 753.7

Note: ¹Fish meal is purchased from American Seafood Company, Seattle, Washington, USA. ²Wheat gluten meal, soybean and rapeseed meal are purchased from Coland Feed Co. Ltd, Wuhan, Hubei, China. ³Oil mixture: soybean oil: fish oil=1:1. ⁴Vitamin premix (mg/kg diet): vitamin A, 1.65; vitamin D, 0.025; vitamin E, 50; vitamin K, 10; ascorbic acid, 100; thiamin, 20; riboflavin, 20; pyridoxine, 20; cyanocobalamine, 0.02; folic acid, 5; calcium pantothenate, 50; inositol, 100; niacin, 100; biotin, 0.1; cellulose, 645.2. ⁵Mineral premix (mg/kg diet): NaCl, 500; MgSO₄·7H₂O, 8155.6; NaH₂PO₄·2H₂O, 12500.0; KH₂PO₄, 16000.0; CaHPO₄·H₂O, 7650.6; FeSO₄·7H₂O, 2286.2; C₆H₁₀CaO₆·5H₂O, 1750.0; ZnSO₄·7H₂O, 178.0; MnSO₄·H₂O, 61.4; CuSO₄·5H₂O, 15.5; CoSO₄·7H₂O, 0.5; KI, 1.5; Corn starch, 753.7

箱60尾鱼。养殖实验持续49d, 每日投喂3次(6:30、11:30和17:30), 实验期间水温为 $(34.9 \pm 0.3)^{\circ}\text{C}$, 溶解氧含量高于5.0 mg/L, 氨氮含量低于0.5 mg/L, 实验光照为自然光照。

1.3 样品采集

在养殖实验结束后, 实验鱼禁食24h。使用麻醉剂MS-222 (60 mg/L, Sigma, USA)将实验鱼麻醉后称总重。每个网箱取2尾鱼用于分析全鱼体组成, 取2尾鱼用于形体指标的测定, 另取3尾鱼进行样品采集。用肝素钠溶液(0.2%)润过的注射器从实验鱼尾部静脉采血, 将血液3000×g离心10min得到血浆, 保存于 -80°C 冰箱中用于后续分析; 随后, 将实验鱼解剖取肌肉组织置于液氮中速冻, 然后转入 -80°C 冰箱中保存。

1.4 样品分析

饲料和全鱼的生化组成测定参考AOAC^[18]的方法: 饲料水分测定采用失重法, 在 105°C 下烘干至恒重; 全鱼的初样和末样在高压蒸汽灭菌锅(ES-315, Tomy Kogyo Co., Japan)中 120°C 蒸30min, 随后捣烂混匀后置于 70°C 烘干, 粉碎成粉末状后于 105°C 下烘干至恒重, 基于失重法计算水分含量。粗蛋白测定采用凯氏定氮法(Kjeltec Auto Analyzer 4800, FOSS Company, USA); 粗脂肪测定采用索氏抽提法(Soxtec System HT Tecator, Extraction Unit, Hoganas, Sweden); 灰分测定采用马福炉(中国湖北英山县建力电炉制造厂) 550°C 燃烧法。

饲料和肌肉的氨基酸组成、血浆游离氨基酸的含量由全自动氨基酸分析仪(Membra Pure, Bodenheim, Germany)检测。取50 mg冷冻干燥后的饲料样品或者肌肉样品于氨基酸消解管中, 加入10 mL盐酸(6 mol/L)后在烘箱中消解24h (110°C), 过滤后用超纯水定容至50 mL。取1 mL溶液进行氮气吹干脱酸, 随后加入1 mL超纯水溶解, 用0.22 μm 滤膜过滤后进行上机检测。取0.4 mL血浆样品加入0.1 mL 10%磺基水杨酸溶液混合均匀后置于 4°C 冷藏60min, 然后15000×g离心15min后取200 μL 上清液, 加入800 μL 超纯水, 用0.22 μm 滤膜过滤后进行上机检测。血浆葡萄糖、甘油三酯和胆固醇含量采用商品试剂盒进行测试(南京建成生物工程研究所, 中国南京)。

对肌肉组织中相关基因的表达量进行测定分析。使用TRIzol (Invitrogen, USA)提取肌肉组织的总RNA, 通过1%琼脂糖凝胶电泳检测总RNA的完整性, 通过Nanodrop 2000超微量分光光度计(Thermo Fisher Scientific, USA)检测总RNA的浓度和质量。使用M-MLV逆转录酶(Promega, USA)将RNA

反转录成cDNA, 采用SYBR[®] Green I Master荧光染色剂(Roche, Switzerland)在罗氏 Light Cycle 480 II PCR仪(Roche, Germany)上进行实时荧光定量反应, 测定目标基因的表达量, 以*gapdh*和*rpl7*为内参基因, 目标基因和内参基因的引物序列见表2。目标基因的相对表达量参照Pfaffl的方法进行计算^[19]。

1.5 数据分析

使用统计软件SPSS 25.0对实验数据进行统计分析, 数据以平均数±标准误表示。对实验数据进行方差齐性检验(Levene homogeneity of variance test), 用单因素方差分析(One-way ANOVA)和Duncan多重比较对数据进行分析, $P < 0.05$ 表示差异显著。

2 结果

2.1 生长性能和形体指标

异育银鲫“中科5号”幼鱼摄食不同赖氨酸水平饲料后生长性能与形体指标见表3。饲料中添加不同水平的赖氨酸对异育银鲫“中科5号”的摄食率、特定生长率、饲料效率、蛋白沉积率、脂肪沉积率没有显著性影响($P > 0.05$)。饲料中添加2%赖氨酸对异育银鲫“中科5号”的肥满度、脏体比和肝体比没有显著性影响($P > 0.05$)。饲料中添加4%赖氨酸组对肥满度、脏体比没有显著影响($P > 0.05$), 但是显著降低了异育银鲫“中科5号”的肝体比($P < 0.05$)。

2.2 全鱼和肌肉的基本组分和氨基酸组成

异育银鲫“中科5号”幼鱼摄食不同赖氨酸水平饲料后全鱼和肌肉的基本组分见表4。饲料中添加2%和4%的赖氨酸对全鱼水分、灰分和脂肪含量没有显著影响($P > 0.05$)。饲料中添加2%赖氨酸组, 全鱼蛋白质含量显著高于其他组($P < 0.05$)。如表4所示, 饲料中添加2%赖氨酸对背肌的蛋白质和脂肪、水分含量没有显著影响($P > 0.05$)。饲料中添加4%赖氨酸组对背肌蛋白质和水分含量没有显著影响($P > 0.05$), 但是显著降低背肌脂肪含量($P < 0.05$)。

异育银鲫“中科5号”幼鱼摄食不同赖氨酸水平饲料后全鱼和肌肉的氨基酸组成见表5。饲料中添加2%赖氨酸组, 全鱼总氨基酸含量、总必需氨基酸含量显著高于其他两个处理组($P < 0.05$), 其中赖氨酸、精氨酸、异亮氨酸、亮氨酸、苯丙氨酸、苏氨酸和缬氨酸等7种必需氨基酸含量显著高于其他各组($P < 0.05$)。肌肉氨基酸组成在各组间无显著性差异($P > 0.05$)。

2.3 血浆葡萄糖、甘油三酯和总胆固醇含量及游离氨基酸含量

异育银鲫“中科5号”幼鱼摄食不同赖氨酸水平饲料后血浆葡萄糖、甘油三酯和总胆固醇含量见

表 2 实时荧光定量引物

Tab. 2 Primers used for qPCR

基因名缩写 Acronym	引物序列 Prime sequence	产物大小 Amplicon size (bp)	登录号 Accession No.
<i>gprc6a</i>	TCAGGGGCTTGCTG GAAC CTGAATGGTTGCTTT GACTCG	170	XM_026283875
<i>β-arrestins</i>	ATCATCATCACCCGC ACACATA GAAGCTTCCGTTTGC TATCCAC	99	XM_052616007
<i>erk</i>	GTGGAACGGAGGAA AGCGTG GCAAATGAACAGAC GAGCGAG	188	XM_052551292
<i>rheb</i>	TTCTTTGGTTTCGAC GTTCCAC ACAAACTGTATCGTG AGGGAGG	118	XM_052599328
<i>tsc2</i>	CTCTCTGCTCCCCTG AAACAC AGATGGGTTGAGAC GCTACG	144	XM_052546044
<i>tor</i>	CGAATCAGGACCTAT GAAGAAA AGCAAGACCACACT AAGACGCC	126	XM_052603534
<i>s6k1</i>	TCCACCCCTACCTCA CACAAG GAATCAAACCTGGCTC ACATCG	217	XM_052576553
<i>4ebp2</i>	CACTTTATTCTCCAC CACCC GATGTTGTTAGCCTC ATTCTT	175	XM_052612896
<i>EIF4E</i>	GACCTTGACCGCTTC TGGAT AGCTCCACACACGTC TTCAC	84	XM_052613501
<i>myod</i>	CTCTGACGGCATGAT GGATTTC CAGACAATCCAAACT CGACACC	139	XM_026239323
<i>myf5</i>	ACCAGTCTACCACGT CCTGT TTCAGCCAAGATTTT GAGGCAA	199	XM_052572730
<i>myog</i>	GGGGGCTTAATTTCC AGACAGA GAATGTCGTATGTTT CGGCAGG	122	XM_052609869
<i>mstn</i>	CGCAAGACACTGTGC AATAGAA TACATCCACGTAACG TTGGACT	128	XM_026271441
<i>mylc</i>	CCAGATTCCATGATG GCAGG AATGGCATCCAAGA ATGGAC	158	XM_026271459
<i>pax7a</i>	GCCTCTTCCGTTAGC TC GTGTAGATGTCAGGG TAGTG	290	XM_026275167
<i>gapdh</i>	AAGGCTGTGGGCAA AGTCA GCACATCCCCGTTGA AATC	229	XM_052578517
<i>rpl7</i>	TGGTCGTTACGGAAT CATCTGC CCCTCAACAAAGTGG GTGGTC	153	XM_059563610

图 1, 各组间无显著性差异($P>0.05$)。异育银鲫“中科5号”幼鱼摄食不同赖氨酸水平饲料后血浆游离

表 3 饲料中添加赖氨酸对异育银鲫“中科5号”生长性能的影响
Tab. 3 Effects of dietary lysine on growth performances of gibel carp “CAS V”

	饲料Diet		
	LL	ML	HL
初始体重 IBW ¹ (g)	6.68±0.02	8.71±0.02	8.69±0.43
终末体重 FBW ² (g)	66.35±1.76	70.51±3.38	69.46±4.29
摄食率 FR ³ (%BW/d)	3.18±0.07	3.22±0.19	3.12±0.07
特定生长率 SGR ⁴ (%/d)	4.15±0.05	4.26±0.09	4.23±0.12
饲料效率 FE ⁵ (%)	97.89±2.83	97.67±7.64	100.26±5.16
蛋白沉积率 PRE ⁶ (%)	42.51±2.00	43.57±5.12	43.39±3.07
脂肪沉积率 LRE ⁷ (%)	84.31±7.82	74.15±6.63	91.04±11.21
肥满度 CF ⁸ (g/cm ³)	3.38±0.02	3.34±0.03	3.35±0.02
肝体比 HSI ⁹	4.99±0.40 ^a	4.69±0.14 ^a	3.88±0.15 ^b
脏体比 VSI ¹⁰	11.36±0.35	10.36±0.03	10.27±0.38

注: 表中所示数据表示为平均值±标准误, 同行数值上标英文字母不同则表示差异性显著($P<0.05$); 下同; ¹初始体重; ²终末体重; ³摄食率 FR (% BW/d)=100×干物质摄食量/[天数×(初始体重+终末体重)/2]; ⁴特定生长率 SGR (%/d)=100×[ln(末重)-ln(初重)]/天数; ⁵饲料效率 FE (%)=(100×鱼体总增重)/摄食量; ⁶蛋白沉积率 PRE (%)=(100×鱼体蛋白质沉积量)/蛋白质摄入量; ⁷脂肪沉积率 LRE (%)=(100×鱼体脂肪沉积量)/脂肪摄入量; ⁸肥满度 CF (g/cm³)=100×体重/体长³; ⁹肝体比 HSI=100×肝脏重/体重; ¹⁰脏体比 VSI=100×内脏重/体重

Note: Values are presented as mean±SE ($n=3$). Values in the same row with different superscript letters are significantly different ($P<0.05$); The same applies below. ¹IBW: Initial body weight; ²FBW: Final body weight; ³Feeding rate (FR, % BW/d)=100×dry feed intake/[days×(IBW+FBW)/2]; ⁴Specific growth rate (SGR, %/d)=100×[ln (final weight) – ln (initial weight)]/days; ⁵Feed efficiency (FE, %)=(100×fresh body weight again)/dry feed intake; ⁶Protein retention efficiency (%)=(100×body retained protein)/protein intake; ⁷Lipid retention efficiency (%)=(100×body retained lipid)/lipid intake; ⁸Condition factor (g/cm³)=100×(body weight)/(body length)³; ⁹Hepatosomatic index=100×liver weight/whole body weight; ¹⁰Viscerosomatic index=100×viscera weight/whole body weight

表 4 饲料中添加赖氨酸对异育银鲫“中科5号”全鱼和肌肉生化组成的影响

Tab. 4 Effects of dietary lysine on chemical composition in whole body and muscle of gibel carp “CAS V”

指标Index	饲料Diet		
	LL	ML	HL
全鱼Whole body			
粗蛋白Crude protein (%)	14.75±0.18 ^b	15.23±0.08 ^a	14.69±0.11 ^b
粗脂肪Crude lipid (%)	6.70±0.45	6.11±0.14	6.48±0.63
灰分Ash (%)	3.73±0.06	3.76±0.04	3.72±0.05
水分Moisture (%)	72.20±0.90	72.51±0.26	72.68±0.87
肌肉Muscle			
粗蛋白Crude protein (%)	22.21±0.07	22.56±0.14	22.41±0.35
粗脂肪Crude lipid (%)	1.61±0.13 ^a	1.52±0.08 ^a	1.17±0.10 ^b
水分Moisture (%)	74.67±0.23	74.37±0.23	74.71±0.54

氨基酸含量见表 6, 血浆游离氨基酸组成没有受到饲料赖氨酸水平的显著影响($P>0.05$)。

2.4 肌肉中相关基因表达

异育银鲫“中科5号”幼鱼摄食不同赖氨酸水平饲料后肌肉中赖氨酸感知和应答相关基因表达量见图 2, *gprc6a*、*β-arrestins*、*erk*、*rheb*、*tsc2*、*tor*、*s6k1*、*4ebp2*、*eif4e*等基因表达量没有受到显著影响($P>0.05$)。

异育银鲫“中科5号”幼鱼摄食不同赖氨酸水平

饲料后肌肉生长相关基因表达量见图 3, 肌肉组织中*myod*、*myf5*、*mstn*、*mylc*和*pax7α*的相对表达量没有显著性差异($P>0.05$), 4%赖氨酸组肌肉*myog*的表达量高于2%赖氨酸组($P<0.05$)。

3 讨论

3.1 低鱼粉饲料添加赖氨酸对异育银鲫“中科5号”幼鱼生长性能的影响

赖氨酸是鱼类的必需氨基酸之一。已有研究

表 5 饲料中添加赖氨酸对异育银鲫“中科5号”全鱼和肌肉氨基酸组成的影响(g/kg干物质)

Tab. 5 Effects of dietary lysine on amino acids profiles in whole body and muscle of gibel carp “CAS V” (g/kg dry matter)

指标Index	全鱼Whole body			肌肉Muscle		
	LL	ML	HL	LL	ML	HL
必需氨基酸Essential amino acids						
赖氨酸 Lys	31.80±1.51 ^b	37.70±0.63 ^a	32.05±1.43 ^b	76.15±3.57	78.18±0.77	73.77±3.27
精氨酸 Arg	26.87±1.02 ^b	31.18±0.44 ^a	27.17±1.65 ^b	47.58±2.37	49.04±0.27	44.57±2.16
组氨酸 His	7.33±0.56 ^{ab}	8.46±0.15 ^a	7.03±0.21 ^b	15.26±1.27	17.69±0.18	15.65±0.50
异亮氨酸 Ile	16.51±0.73 ^b	19.84±0.31 ^a	16.58±0.88 ^b	35.61±1.38	36.11±0.13	33.25±1.57
亮氨酸 Leu	28.82±1.44 ^b	34.90±0.65 ^a	29.75±1.72 ^b	61.45±2.51	63.63±0.35	58.59±2.32
苯丙氨酸 Phe	16.05±1.02 ^b	19.34±0.51 ^a	16.27±0.95 ^b	33.87±1.75	34.27±0.16	31.46±1.09
苏氨酸 Thr	17.27±0.71 ^b	20.65±0.45 ^a	18.14±0.82 ^b	34.98±1.98	35.19±0.31	32.72±1.46
缬氨酸 Val	18.63±0.98 ^b	22.44±0.58 ^a	18.67±0.94 ^b	37.28±1.77	38.27±0.38	35.35±1.49
蛋氨酸 Met	10.92±0.61	12.36±0.35	11.23±0.73	21.42±0.88	21.07±0.15	21.01±0.94
总必需氨基酸TEAA	174.19±8.52 ^b	206.86±3.08 ^a	176.89±9.19 ^b	363.60±17.17	373.43±1.55	346.36±14.47
非必需氨基酸 Non-essential amino acids						
天冬氨酸 Asp	37.88±1.73 ^b	45.18±1.03 ^a	39.24±1.94 ^b	78.87±3.88	80.61±0.47	75.64±3.31
丝氨酸 Ser	15.49±0.58 ^b	18.50±0.74 ^a	16.63±0.83 ^{ab}	27.69±1.11	27.87±0.28	26.75±0.77
谷氨酸 Glu	53.93±2.23 ^b	64.43±1.91 ^a	56.00±2.85 ^b	111.85±4.60	116.03±0.39	109.10±4.82
甘氨酸 Gly	34.50±1.34 ^b	40.45±0.69 ^a	34.24±1.79 ^b	35.11±1.72	35.48±0.25	35.34±3.05
丙氨酸 Ala	27.45±1.35 ^b	32.60±0.61 ^a	27.84±1.39 ^b	46.24±2.20	46.99±0.26	44.63±2.34
酪氨酸 Tyr	10.48±0.56 ^b	12.59±0.32 ^a	11.12±0.45 ^{ab}	24.36±1.03	24.62±0.34	23.51±0.76
脯氨酸 Pro	20.61±1.08 ^b	25.02±0.55 ^a	21.59±1.33 ^{ab}	23.29±1.42	23.96±0.35	23.20±1.71
胱氨酸 Cys	0.38±0.24	0.82±0.25	0.44±0.22	1.07±0.35	1.15±0.46	1.00±0.07
总非必需氨基酸 TNEAA	180.13±7.97 ^b	214.58±5.37 ^a	185.50±9.35 ^b	325.19±14.62	332.76±0.76	315.97±14.68
总氨基酸TAA	354.32±16.48 ^b	421.43±8.37 ^a	362.4±18.49 ^b	688.79±31.73	706.20±2.31	662.33±29.15

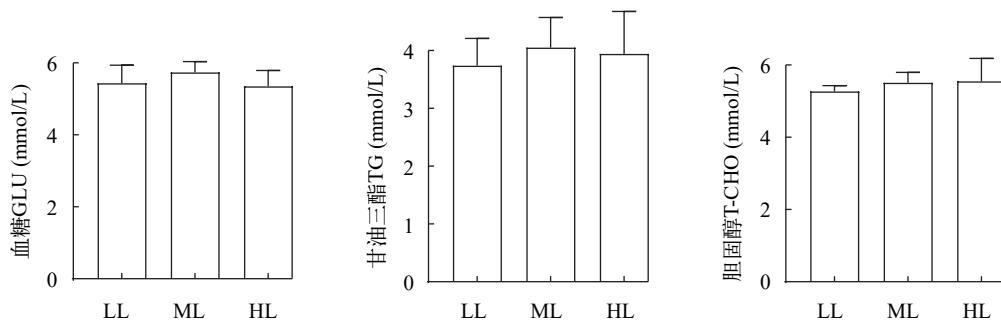


图 1 饲料中添加赖氨酸对异育银鲫“中科5号”血浆生化指标的影响

Fig. 1 Effects of dietary lysine on plasma biochemical indices of gibel carp “CAS V”

数值表示为平均值±标准误($n=6$); 下同

Values are expressed as means±SE ($n=6$); The same applies below

表 6 饲料中添加赖氨酸对异育银鲫“中科5号”幼鱼血浆游离氨基酸的影响

Tab. 6 Effects of dietary lysine on plasma free amino acids profile of gibel carp “CAS V” ($\mu\text{mol/mL}$)

指标Index	饲料Diet		
	LL	ML	HL
必需氨基酸 Essential amino acids			
赖氨酸 Lys	2.68±0.26	2.54±0.30	2.81±0.23
精氨酸 Arg	2.94±0.12	3.07±0.15	3.28±0.16
组氨酸 His	1.20±0.14	1.20±0.14	1.21±0.07
异亮氨酸 Ile	2.15±0.19	2.26±0.31	2.55±0.17
亮氨酸 Leu	1.84±0.12	1.81±0.13	2.02±0.10
苯丙氨酸 Phe	0.37±0.03	0.40±0.04	0.33±0.04
苏氨酸 Thr	2.83±0.14	2.72±0.20	3.01±0.26
缬氨酸 Val	3.80±0.32	3.82±0.47	4.32±0.32
蛋氨酸 Met	0.33±0.04	0.27±0.03	0.24±0.02
总必需氨基酸 TEAA	18.14±1.06	18.10±1.48	19.76±0.94
非必需氨基酸 Non-essential amino acids			
天冬氨酸 Asp	0.15±0.03	0.11±0.02	0.17±0.03
丝氨酸 Ser	2.64±0.22	2.25±0.22	2.73±0.28
谷氨酸 Glu	0.68±0.06	0.61±0.09	0.73±0.05
甘氨酸 Gly	2.59±0.44	2.11±0.17	2.51±0.26
丙氨酸 Ala	3.38±0.29	3.14±0.20	3.66±0.29
酪氨酸 Tyr	0.87±0.11	0.81±0.54	0.89±0.11
脯氨酸 Pro	0.32±0.98	0.39±0.15	0.39±0.10
胱氨酸 Cys	0.10±0.01	0.08±0.01	0.06±0.01
总非必需氨基酸 TNEAA	10.41±1.03	9.11±0.71	10.76±0.93
总氨基酸 TAA	28.55±1.98	27.21±2.18	30.52±1.84

注: 表中所示数据表示为平均值±标准误($n=6$)

Note: Values are presented as mean±SE ($n=6$)

表明, 饲料含有适宜水平的赖氨酸会显著提高实验鱼的生长性能和鱼体蛋白质含量, 赖氨酸缺乏或者过量会导致鱼体生长受阻^[20–22]。前期研究表明异育银鲫幼鱼对赖氨酸的需求量占饲料的3.27%, 饲料中赖氨酸含量为1.82%、3.8%和4.82%组的特定生长率和饲料利用无显著性差异^[23]。随着饲料中赖氨酸水平的增加, 养成中期异育银鲫“中科3号”的特定生长率、蛋白沉积率及能量沉积率逐渐升高, 在1.83%组达到最大之后逐渐下降^[24]。在本研究中, 在低鱼粉饲料中添加不同水平赖氨酸(饲料赖氨酸实测含量分别为1.77%、3.94%和6.82%)对异育银鲫“中科5号”的摄食率、特定生长率、饲料效率、蛋白沉积率、脂肪沉积率没有显著性影响。在黄颡鱼里有相似的结果, 可能是因为基础饲料中的赖氨酸含量满足了实验鱼的最低需要量, 并且最高添加量没有达到抑制生长的水平^[25]。实验鱼的遗传背景^[26]、饲料成分组成^[27]、投喂模式^[28]等因素都会影响饲料赖氨酸水平对实验鱼生长性

能的作用。

3.2 低鱼粉饲料添加赖氨酸对异育银鲫“中科5号”幼鱼全鱼和肌肉的营养组成及血浆生理生化指标影响

在很多鱼类中已报道, 饲料中赖氨酸含量提高可能会提高鱼体蛋白含量, 降低鱼体脂肪含量^[29–31]。本研究中有类似的结果, 饲料中添加2%赖氨酸组, 全鱼蛋白质含量显著高于其他组, 同时全鱼赖氨酸、精氨酸、异亮氨酸、亮氨酸、苯丙氨酸、苏氨酸和缬氨酸等7种必需氨基酸含量、总必需氨基酸、总氨基酸含量显著高于其他两个处理组。鱼体组织氨基酸和蛋白质的沉积与饲料赖氨酸水平有一定的相关性, 饲料中高赖氨酸水平的添加有助于增强饲料的氨基酸平衡性, 有利于提高鱼体的蛋白合成能力^[32, 33]。饲料中缺乏特定必需氨基酸会提高鱼体组织氨基酸氧化率^[34], 当饲料赖氨酸水平高于适宜水平时, 肌肉中总氨基酸含量和赖氨酸含量显著下降, 表明赖氨酸过量可能会提高自身和其他氨基酸的分解代谢, 在黄颡鱼^[35]和黑鲷(*Sparus macrocephalus*)^[36]等鱼类的研究中也发现有相似的结果。赖氨酸还能参与肉碱的合成, 在长链脂肪酸的 β 氧化过程中行使重要功能。在饲料中单独添加赖氨酸、肉碱或两者都添加可以显著降低斑点叉尾鲷(*Ictalurus punctatus*)鱼体脂含量, 饲料赖氨酸含量不足会引起肉碱合成减少, 从而抑制脂肪的氧化代谢, 诱导脂肪在鱼体内沉积^[37]。在本研究中, 饲料中添加4%赖氨酸显著降低了背肌的脂肪含量, 同时显著降低了肝体比, 这与斑点叉尾鲷的结果一致。前期在异育银鲫幼鱼实验中, 血浆游离氨基酸在摄食赖氨酸饲料6h后与对照组无显著性差异^[24]。在本研究中, 取样时间为摄食后24h, 因此血浆游离氨基酸组成无差异可能是与取样时间有关。异育银鲫“中科3号”^[24]和大菱鲆^[38]等鱼类中的研究显示, 饲料氨基酸不平衡可能会对糖脂代谢造成显著影响。在本研究中, 血浆葡萄糖、甘油三酯和胆固醇的含量均没有受到饲料赖氨酸水平的影响, 与红鳍东方鲀(*Takifugu rubripes*)的结果一致^[39]。

3.3 低鱼粉饲料添加赖氨酸对异育银鲫“中科5号”幼鱼肌肉中相关基因表达的影响

GPRC6A是一种重要的氨基酸感知受体, 可以特异性识别赖氨酸^[5]。在本研究中, 肌肉中赖氨酸感知基因 $gprc6a$ 的相对表达量在各组间没有显著差异, 这与血浆游离氨基酸的结果一致。GPRC6A可以通过 β -arrestins调节ERK信号转导途径, ERK通过抑制Tsc2-Rheb通路激活mTOR通路^[6–9]。在本研究中, 肌肉中赖氨酸应答相关基因 β -arrestins、 erk 、 $rheb$ 、 $tsc2$ 和 tor 的相对表达量没有受到饲料

的影响,与*gprc6a*的相对表达量保持一致。mTOR可以通过调控其下游信号分子核糖体蛋白S6激酶(Ribosomal protein S6 kinase 1, S6K1)、真核翻译起始因子4E (Eukaryotic translation initiation factor 4E, eIF4E)和真核翻译因子4E结合蛋白2 (Eukaryotic translation initiation factor 4E binding proteins, 4EBP2)控制着细胞的总体翻译能力^[40]。本研究中*s6k1*、*4ebp2*和*eif4e*的相对表达量在各组间没有差异,与*tor*的结果一致。杂交石斑鱼(*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂)的研究结果表明,当饲料赖氨酸含量高于最适需求量,肝脏中*tor*和*s6k1*的表达量在饲料组间无显著差异^[41],所以本实验中mTOR通路相关基因在饲料组间无差异可能是因为基础饲料中的赖氨酸水平已经满足实验鱼的基本需求,这也可能是肌肉蛋白质含量没有显著上升的原因。

鱼类的生长和肌纤维的生长发育密切相关,生肌因子5 (Myogenic factor 5, Myf5)和肌分化因子(Myogenic differentiation, MyoD)主要在肌肉成肌细胞增殖的过程中表达,肌细胞生成素(Myogenin, MyoG)主要在肌肉分化过程中起作用^[42-44]。草鱼(*Ctenopharyngodon idellus*)肌肉中*myog*和*myf5*基因表达量随着饲料赖氨酸水平的升高而显著升高,并在赖氨酸水平为12.7 g/kg时达到最大,然后逐渐降低;在饲料赖氨酸含量从6.0增加到10.3 g/kg时,肌肉中*myod*基因表达量显著升高,但没有进一步增加;这说明饲料赖氨酸水平在一定范围内(6.0—12.7 g/kg)能够显著提高草鱼肌肉中生肌因子*myod*、*myf5*和*myog*的基因表达量^[45]。本研究中的异育银鲫“中科5号”幼鱼摄食不同赖氨酸水平饲料后肌肉组织中*myod*、*myf5*的相对表达量没有显著性差异,可能是饲料中赖氨酸含量不在可有效提高*myod*、*myf5*基

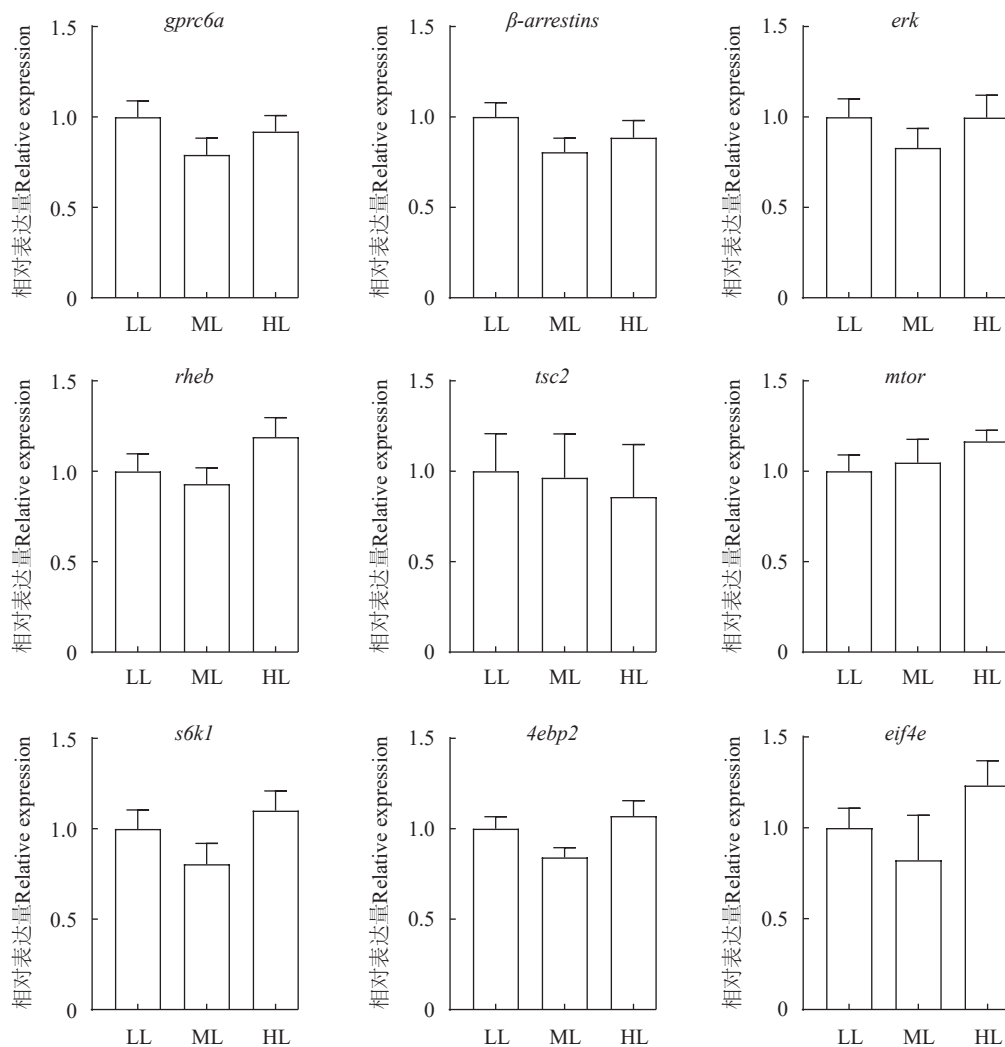


图2 饲料中添加赖氨酸对异育银鲫“中科5号”肌肉中赖氨酸感知和应答相关基因相对表达量的影响

Fig. 2 Effects of dietary lysine on the mRNA levels of gene involved in sensing and responding to lysine in the muscle of gibel carp “CAS V”

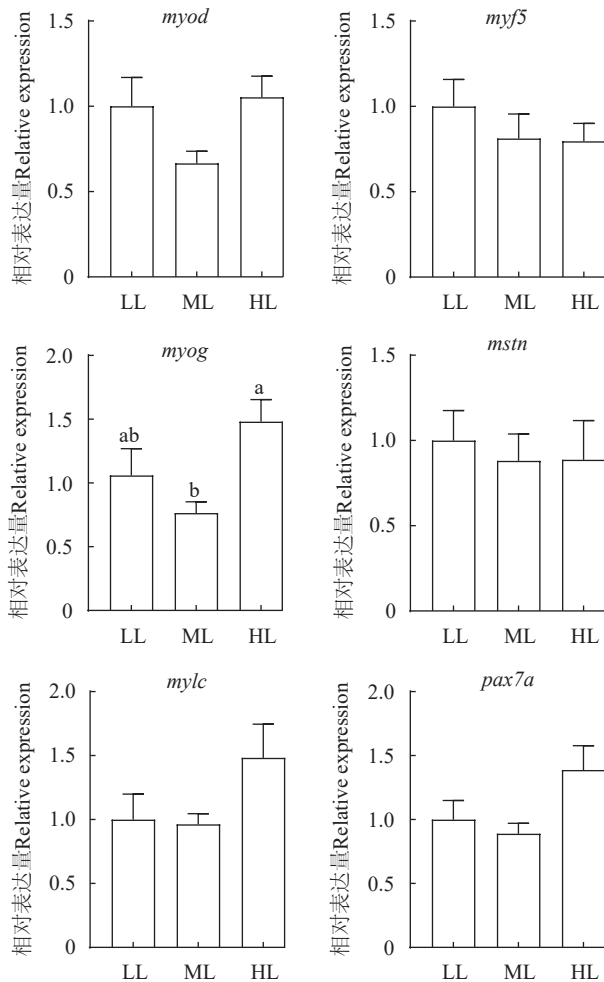


图3 饲料中添加赖氨酸对异育银鲫“中科5号”肌肉中调控肌纤维生长和发育相关基因相对表达量的影响

Fig. 3 Effects of dietary lysine on the mRNA levels of gene involved in regulating myofiber growth and development in the muscle of gibel carp “CAS V”

各处理组不同字母表示差异显著($P < 0.05$)

Different letters indicate significant differences among groups ($P < 0.05$)

因表达量的范围内。同时,本研究中4%赖氨酸组肌肉*myog*的表达量高于2%赖氨酸组,说明饲料中添加4%赖氨酸在一定程度上促进了肌原细胞的分化。肌肉生长抑制素(Myostatin, MSTN)是肌肉生长的负调控因子,是通过结合受体将信号传递到核内,抑制*myod*基因的表达^[46]。在本研究中,*mstn*的相对表达量在各组间没有显著性差异,在吉富罗非鱼(*Oreochromis niloticus*)中也有相似的结果^[47],同时与*myod*的结果保持一致。肌球蛋白轻链(Myosin light chain, MyLC)是肌球蛋白的重要组成部分^[48],骨骼肌的生长和发育还有其他的调节因子包括配对盒基因(Paired box gene, PAX)等参与其中^[49]。在本研究中,异育银鲫“中科5号”幼鱼摄食不同赖氨酸

酸水平饲料后肌肉组织中*mylc*和*pax7a*的相对表达量没有显著性差异。肌肉的生长是一个复杂的过程,赖氨酸对异育银鲫肌纤维生长的作用机制有待于进一步探究。

综上所述,低鱼粉饲料中添加2%和4%赖氨酸对异育银鲫“中科5号”的生长性能无显著影响,但是添加2%赖氨酸可以有效提高鱼体蛋白质和总必需氨基酸的含量,具体的调控机制有待进一步的探究,研究结果可为异育银鲫“中科5号”的精准配方设计特别是低鱼粉饲料配方设计提供数据支撑。

(作者声明本文符合出版伦理要求)

参考文献:

- [1] Han D, Shan X, Zhang W, *et al.* A revisit to fishmeal usage and associated consequences in Chinese aquaculture [J]. *Reviews in Aquaculture*, 2018, **10**(2): 493-507.
- [2] Fu B, Fu L L, Cao J M, *et al.* Effects of four additives added to low fish meal feed on growth performance, serum biochemical indexes and physical and chemical indexes of breeding water body of *Lateolabrax japonicus* [J]. *Feed Industry*, 2022, **43**(6): 45-51. [符兵, 伏彬龙, 曹俊明, 等. 低鱼粉饲料中添加4种添加剂对花鲈生长性能、血清生化指标及养殖水体理化指标的影响 [J]. 饲料工业, 2022, **43**(6): 45-51.]
- [3] Sun S K, Gao Y N, Ma S, *et al.* Effect of adding microcapsule lysine and methionine on growth and biochemical parameters of *Cyprinus carpio* in feed [J]. *Feed Research*, 2023, **46**(12): 51-55. [孙树奎, 高亚楠, 马爽, 等. 饲料中添加微囊赖氨酸、蛋氨酸对鲤鱼生长及生化指标的影响 [J]. 饲料研究, 2023, **46**(12): 51-55.]
- [4] Jewell J L, Russell R C, Guan K L. Amino acid signalling upstream of mTOR [J]. *Nature Reviews Molecular Cell Biology*, 2013, **14**(3): 133-139.
- [5] Wellendorph P, Bräuner-Osborne H. Molecular basis for amino acid sensing by family C G-protein-coupled receptors [J]. *British Journal of Pharmacology*, 2009, **156**(6): 869-884.
- [6] Winter J N, Fox T E, Kester M, *et al.* Phosphatidic acid mediates activation of mTORC1 through the ERK signaling pathway [J]. *Cell Physiology*, 2010, **299**(2): C335-C344.
- [7] Winter J N, Jefferson L S, Kimball S R. ERK and Akt signaling pathways function through parallel mechanisms to promote mTORC1 signaling [J]. *American Journal of Physiology Cell Physiology*, 2011, **300**(5): C1172-C1180.
- [8] Ye R, Pi M, Nooh M M, *et al.* Human GPRC6A mediates testosterone induced mitogen activated protein kinases and mTORC1 signaling in prostate cancer cells [J]. *Molecular Pharmacology*, 2019, **95**(5): 563-572.
- [9] Zhang D, Han S, Wang S, *et al.* cPKCγ-mediated down-regulation of UCHL1 alleviates ischaemic neuronal

- injuries by decreasing autophagy via ERK-mTOR pathway [J]. *Journal of Cellular and Molecular Medicine*, 2017, **21**(12): 3641-3657.
- [10] Mai K S. History and prospect of aquaculture nutrition and aquafeed industry of China [J]. *Feed Industry*, 2020, **41**(1): 2-6. [麦康森. 中国水产动物营养研究与饲料工业的发展历程与展望 [J]. 饲料工业, 2020, **41**(1): 2-6.]
- [11] Wang X, Proud C G. The mTOR pathway in the control of protein synthesis [J]. *Physiology*, 2006(21): 362-369.
- [12] Jiang H, Bian F, Zhou H, *et al.* Nutrient sensing and metabolic changes after methionine deprivation in primary muscle cells of turbot (*Scophthalmus maximus* L.) [J]. *The Journal of Nutritional Biochemistry*, 2017(50): 74-82.
- [13] Qin Q, Cao X F, Dai Y J, *et al.* Effects of dietary protein level on growth performance, digestive enzyme activity, and gene expressions of the TOR signaling pathway in fingerling *Pelteobagrus fulvidraco* [J]. *Fish Physiology and Biochemistry*, 2019, **45**(5): 1747-1757.
- [14] Tu Y, Xie S, Han D, *et al.* Dietary arginine requirement for gibel carp (*Carassis auratus gibelio* var. CAS III) reduces with fish size from 50 g to 150 g associated with modulation of genes involved in TOR signaling pathway [J]. *Aquaculture*, 2015(449): 37-47.
- [15] Li H, Ji S, Yuan X, *et al.* Eicosapentaenoic acid (EPA) improves grass carp (*Ctenopharyngodon idellus*) muscle development and nutritive value by activating the mTOR signaling pathway [J]. *Fish Physiology Biochemistry*, 2024(50): 687-703.
- [16] Ministry of Agriculture of the People's Republic of China. Chinese Fishery Statistical Yearbook [M]. Beijing: Chinese Agricultural Press, 2023: 25. [中华人民共和国农业农村部. 中国渔业统计年鉴 [M]. 北京: 中国农业出版社, 2023: 25.]
- [17] Gui J F, Zhou L, Zhang X J. Research advances and prospects for fish genetic breeding [J]. *Bulletin of Chinese Academy of Sciences*, 2018, **33**(9): 932-939. [桂建芳, 周莉, 张晓娟. 鱼类遗传育种发展现状与展望 [J]. 中国科学院院刊, 2018, **33**(9): 932-939.]
- [18] AOAC, Official Methods of Analysis of the Association of Official Analytical Chemists [M]. 17th ed. Association of Official Analytical Chemists, Arlington, VA, USA., 2003.
- [19] Pfaffl M W. A new mathematical model for relative quantification in Real-time RT-PCR [J]. *Nucleic Acids Research*, 2001, **29**(9): e45.
- [20] Azizi S, Ali Nematollahi M, Mojazi Amiri B, *et al.* Lysine and leucine deficiencies affect myocytes development and IGF signaling in gilthead sea bream (*Sparus aurata*) [J]. *PLoS One*, 2016, **11**(1): e0147618.
- [21] Niu X T, Zuo Y N, Zhang J S, *et al.* Effects of dietary lysine level on growth, feed utilization, serum biochemical indexes, lysine metabolizing enzyme activity and related gene expression of *Leuciscus brandti* [J]. *Journal of Fisheries of China*, 2019, **43**(10): 2154-2165. [牛小天, 左亚南, 张家松, 等. 饲料中赖氨酸水平对勃氏雅罗鱼生长、饲料利用、血液生化指标、赖氨酸代谢酶活性及相关基因表达的影响 [J]. 水产学报, 2019, **43**(10): 2154-2165.]
- [22] Wei Y, Li B, Xu H, *et al.* Effects of lysine and leucine in free and different dipeptide forms on the growth, amino acid profile and transcription of intestinal peptide, and amino acid transporters in turbot (*Scophthalmus maximus*) [J]. *Fish Physiology Biochemistry*, 2020, **46**(5): 1795-1807.
- [23] Zhou X J, Xie S Q, Xie C X, *et al.* The utilization and requirement of dietary lysine for juvenile gibel carp [J]. *Acta Hydrobiologica Sinica*, 2006, **30**(3): 247-255. [周贤君, 解绶启, 谢从新, 等. 异育银鲫幼鱼对饲料中赖氨酸的利用及需要量研究 [J]. 水生生物学报, 2006, **30**(3): 247-255.]
- [24] Tu Y Q. Comparative study on utilization of dietary lysine and arginine in gibel carp of different body sizes [D]. Beijing: University of Chinese Academy of Sciences, 2015. [涂永芹. 不同规格异育银鲫对饲料赖氨酸和精氨酸利用的比较研究 [D]. 北京: 中国科学院大学, 2015.]
- [25] Shen Y, Qiu Q J, Sun L S, *et al.* Effects of dietary arginine /lysine on growth performance, body composition, serum biochemical indices and amino acid deposition rate of all-male yellow catfish [J]. *Chinese Journal of Animal Nutrition*, 2017, **29**(7): 2575-2586. [沈勇, 邱其浚, 孙龙生, 等. 饲料精氨酸与赖氨酸配比对全雄黄颡鱼生长性能、体组成、血清生化指标及氨基酸沉积率的影响 [J]. 动物营养学报, 2017, **29**(7): 2575-2586.]
- [26] Gatrell S K, Silverstein J T, Barrows F T, *et al.* Effect of dietary lysine and genetics on growth and indices of lysine catabolism in rainbow trout (*Oncorhynchus mykiss*) [J]. *Aquaculture Nutrition*, 2017, **23**(5): 917-925.
- [27] Bodin N, Govaerts B, Abboudi T, *et al.* Protein level affects the relative lysine requirement of growing rainbow trout (*Oncorhynchus mykiss*) fry [J]. *The British Journal of Nutrition*, 2009, **102**(1): 37-53.
- [28] Zarate, Lovell, Payne. Effects of feeding frequency and rate of stomach evacuation on utilization of dietary free and protein-bound lysine for growth by channel catfish *Ictalurus punctatus* [J]. *Aquaculture Nutrition*, 1999, **5**(1): 17-22.
- [29] Zhou Q C, Wu Z H, Chi S Y, *et al.* Dietary lysine requirement of juvenile cobia (*Rachycentron canadum*) [J]. *Aquaculture*, 2007, **273**(4): 634-640.
- [30] Zhang C, Ai Q, Mai K, *et al.* Dietary lysine requirement of large yellow croaker, *Pseudosciaena crocea* R [J]. *Aquaculture*, 2008, **283**(1-4): 123-127.
- [31] Deng D, Dominy W, Ju Z Y, *et al.* Dietary lysine requirement of juvenile Pacific threadfin (*Polydactylus sexfilis*) [J]. *Aquaculture*, 2010, **308**(1-2): 44-48.

- [32] Wilson R P, Poe W E. Relationship of whole body and egg essential amino acid patterns to amino acid requirement patterns in channel catfish, *Ictalurus punctatus* [J]. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, 1985, **80**(2): 385-388.
- [33] Ng W K, Hung S S O, Herold M A. Poor utilization of dietary free amino acids by white sturgeon [J]. *Fish Physiology and Biochemistry*, 1996, **15**(2): 131-142.
- [34] Zarate D D, Lovell R T. Free lysine (l-lysine · HCl) is utilized for growth less efficiently than protein-bound lysine (soybean meal) in practical diets by young channel catfish (*Ictalurus punctatus*) [J]. *Aquaculture*, 1997, **159**(1-2): 87-100.
- [35] Cao J M, Chen Y, Zhu X, *et al.* A study on dietary l-lysine requirement of juvenile yellow catfish *Pelteobagrus fulvidraco* [J]. *Aquaculture Nutrition*, 2012, **18**(1): 35-45.
- [36] Zhou F, Shao J, Xu R, *et al.* Quantitative l-lysine requirement of juvenile black sea bream (*Sparus macrocephalus*) [J]. *Aquaculture Nutrition*, 2010, **16**(2): 194-204.
- [37] Burtle G J, Liu Q. Dietary carnitine and lysine affect channel catfish lipid and protein composition [J]. *Journal of the World Aquaculture Society*, 1994, **25**(2): 169-174.
- [38] Huang X, Song X, Wang X, *et al.* Dietary lysine level affects digestive enzyme, amino acid transport and hepatic intermediary metabolism in turbot (*Scophthalmus maximus*) [J]. *Fish Physiology and Biochemistry*, 2022, **48**(4): 1091-1103.
- [39] Zhang Q G, Wang J X, Wei Y L, *et al.* Requirement of lysine in juvenile tiger puffer (*Takifugu rubripes*) [J]. *Chinese Journal of Animal Nutrition*, 2020, **32**(2): 847-855. [张庆功, 王建学, 卫育良, 等. 红鳍东方鲀幼鱼赖氨酸需求量的研究 [J]. *动物营养学报*, 2020, **32**(2): 847-855.]
- [40] Wullschleger S, Loewith R, Hall M N. TOR signaling in growth and metabolism [J]. *Cell*, 2006, **124**(3): 471-484.
- [41] Li X, Wu X, Dong Y, *et al.* Effects of dietary lysine levels on growth, feed utilization and related gene expression of juvenile hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂) [J]. *Aquaculture*, 2019(502): 153-161.
- [42] Kassar-Duchossoy L, Gayraud-Morel B, Gomès D, *et al.* Mrf4 determines skeletal muscle identity in Myf5: Myod double-mutant mice [J]. *Nature*, 2004, **431**(7007): 466-471.
- [43] Johansen K A, Overturf K. Alterations in expression of genes associated with muscle metabolism and growth during nutritional restriction and refeeding in rainbow trout [J]. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 2006, **144**(1): 119-127.
- [44] Ropka-Molik K, Eckert R, Piórkowska K. The expression pattern of myogenic regulatory factors MyoD, Myf6 and Pax7 in postnatal porcine skeletal muscles [J]. *Gene Expression Patterns*, 2011, **11**(1-2): 79-83.
- [45] Tang Y J. Effects of dietary lysine on growth performance and flesh quality of sub-adult grass carp and its mechanism [D]. Ya'an: Sichuan Agricultural University, 2022. [唐延杰. 饲料赖氨酸对生长后期草鱼生长性能与肌肉品质的影响及作用机制 [D]. 雅安: 四川农业大学, 2022.]
- [46] Zhang F, Deng B, Wen J, *et al.* PPAR γ and MyoD are differentially regulated by myostatin in adipose-derived stem cells and muscle satellite cells [J]. *Biochemical and Biophysical Research Communications*, 2015, **458**(2): 375-380.
- [47] Prabu E, Felix N, Uma A, *et al.* Effects of dietary L-lysine supplementation on growth, body composition and muscle-growth-related gene expression with an estimation of lysine requirement of GIFT tilapia [J]. *Aquaculture Nutrition*, 2020, **26**(2): 568-578.
- [48] Hettige P, Tahir U, Nishikawa K C, *et al.* Comparative analysis of the transcriptomes of EDL, psoas, and soleus muscles from mice [J]. *BMC Genomics*, 2020, **21**(1): 808.
- [49] Bhagavati S, Song X, Siddiqui M A Q. RNAi inhibition of Pax3/7 expression leads to markedly decreased expression of muscle determination genes [J]. *Molecular and Cellular Biochemistry*, 2007, **302**(1-2): 257-262.

DIETARY LYSINE SUPPLEMENTATION ON GROWTH PERFORMANCES AND METABOLISM IN JUVENILE GIBEL CARP (*CARASSIUS GIBELIO* VAR. CAS V) FED LOW FISH MEAL DIETS

XU Wen-Jie¹, WANG Yu^{2,3}, WU Li-Yun², DONG Bo^{2,3}, ZHU Xiao-Ming², HAN Dong², LIU Hao-Kun²,
YANG Yun-Xia², ZHANG Zhi-Min², XIE Shou-Qi^{2,3} and JIN Jun-Yan²

(1. College of Food Science and Engineering, Guangdong Ocean University, Yangjiang 529500, China; 2. State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China; 3. University of Chinese Academy of Sciences, Beijing 100049, China)

Abstract: How to improve utilization of protein especially non-fishmeal protein in fish is a problem demanding prompt solution in aquatic nutrition. Studies in mammal showed that G protein coupled receptor family C, group 6, subtype A (GPRC6A) could sense the amino acids, and then regulate the muscle growth via ERK-mTOR signal pathway. But mechanisms of sensing and responding to lysine in fish are still unclear. A 7-week feeding experiment was conducted to investigate the effects of dietary lysine supplementation in low fish meal diets on the growth performances, biochemical composition, nutrient deposition, plasma biochemical indices, and metabolism related gene expression in the muscle of gibel carp (*Carassius gibelio* var. CAS V) initial mean body weight: (8.70±0.03) g. The diet containing 5% fish meal was set as the control group (LL), the other groups were supplemented with 2% (ML) and 4% (HL) lysine, respectively. The results showed that lysine supplementation in low fishmeal diet had no significant effect on the feeding rate (FR), specific growth rate (SGR), feed efficiency (FE), protein retention efficiency (PRE), and lipid retention efficiency (LRE) in the gibel carp ($P>0.05$). Dietary supplement with 2% lysine did not affect the condition factor (CF), hepatosomatic index (HSI), and viscerosomatic index (VSI) in the gibel carp ($P>0.05$). The 4% lysine group had no effect on the CF and VSI, but significantly decreased the HSI ($P<0.05$). The protein content of whole body in ML group was higher than that in other two groups ($P<0.05$), and the total amino acid level and the total essential amino acid level was also higher than LL group and HL group ($P<0.05$). The levels of seven essential amino acids including lysine, arginine, isoleucine, leucine, phenylalanine, threonine, and valine in the whole body was the highest in the fish fed diet supplemented with 2% lysine ($P<0.05$). The lysine supplementation in low fishmeal diet had no significant effect on the levels of protein and moisture in the muscle of gibel carp ($P>0.05$), but the HL diet decrease the lipid contents in the muscle ($P<0.05$). Plasma glucose, triglyceride and cholesterol levels showed no significant difference in the three groups ($P>0.05$). The diets supplemented with lysine did not affect the plasma amino acid profile in gibel carp ($P>0.05$). The mRNA levels of genes involved in sensing and responding to lysine including *gprc6a*, *β-arrestins*, *erk*, *rheb*, *tsc2*, *tor*, *s6k1*, *4ebp2*, and *EIF4E* in the muscle of gibel carp were not affected by the diets ($P>0.05$). The lysine supplementation in low fishmeal diet had no significant effect on relative expression of genes involved in regulating myofiber growth and development including *myod*, *myf5*, *mstn*, *mylc*, and *pax7α* in the muscle of gibel carp ($P>0.05$), but the mRNA levels of *myog* in the HL group was higher than that in the ML group ($P<0.05$). These results suggested that low fish meal diet supplemented with 2% lysine could increase the total essential amino acid level and protein level in the whole body of gibel carp “CAS V”, but the mechanism remains to be fully elucidated, which might offer new insights into improving the feed formula of gibel carp “CAS V”.

Key words: Lysine; Growth performances; Amino acid sensing; Metabolism; *Carassius gibelio* var. CAS V