Hu W G, Shi T, Mao X J, *et al*. Different dietary iron sources on intestinal health of largemouth bass (*Micropterus salmoides*) [J]. *Acta Hydrobiologica Sinica*, 2025, 49(9): 092511. [胡文广, 施涛, 毛香杰, 等. 不同饲料铁源对大口黑鲈肠道健康的影响 [J]. 水生生物学报, 2025, 49(9): 092511.]

不同饲料铁源对大口黑鲈肠道健康的影响

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摘要: 实验旨在探究硫酸亚铁($FeSO_4$)、蛋氨酸螯合铁(Fe-Met)、甘氨酸铁(Fe-Gly)和复合氨基酸螯合铁(Fe-CAA)分别作为饲料中铁源对大口黑鲈(*Micropterus salmoides*)肠道健康的调节作用。以上述4种铁源设计4组适宜铁水平且等氮等脂的饲料(简称为$FeSO_4$、Fe-Met、Fe-Gly和Fe-CAA), 每组设置3个重复, 进行为期70d的大口黑鲈幼鱼养殖实验。在养殖结束后, 测定各组肠道SOD、CAT和T-AOC等抗氧化物酶活性, 并对肠道健康相关基因和菌群进行分析比较。结果表明, $FeSO_4$组的MDA活性显著高于Fe-Gly组和Fe-CAA组(P<0.05), $FeSO_4$组的T-AOC活性则显著低于Fe-Met组和Fe-CAA组(P<0.05), Fe-Met组的CAT水平显著高于其他3组。在基因水平上, $FeSO_4$组的*IL-8*和*Occludin*基因表达显著高于其他3组(P<0.05), Fe-Gly组和Fe-CAA组的*ZO-1*和*Claudin-1*基因表达显著高于$FeSO_4$组和Fe-Met组(P<0.05)。*IL-10*、*IL-6*与*IL-15*的基因表达则未受到铁源的显著影响。进一步进行肠道菌群分析发现, 相较于$FeSO_4$组, Fe-CAA组的菌落多样性显著降低(P<0.05)。在门水平上, 相较于$FeSO_4$组, Fe-CAA组厚壁菌门显著下调, 而变形菌门、梭杆菌门显著上调(P<0.05); 在属水平上, Fe-CAA组邻单胞菌属(*Plesiomonas*)、肠杆菌属(*Enterobacter*)、鲸杆菌属(*Cetobacterium*)等显著上调, 乳球菌属(*Lactococcus*)、气单胞菌属(*Aeromonas*)等显著下调(P<0.05)。对肠道菌群和抗氧化物酶进行相关性分析发现, 肠道MDA活性与大肠志贺氏杆菌(*Escherichia-Shigella*)和肠杆菌(*Enterobacter*)等具有强烈的相关性。肠道T-AOC活性与邻居单胞菌属(*Plesiomonas*)和无色菌属(*Achromobacter*)等也显示出了强烈的相关性。此外, 肠道SOD活性与无色菌属(*Achromobacter*)有强烈的负相关, 肠道GSH活性与链球菌属(*Streptococcus*)也显示出了强烈负相关。但微生物群落功能预测分析结果表明, 两组处理的肠道微生物群落功能上没有显著差异。综上, 有机铁源相较于无机铁源对大口黑鲈的肠道健康及抗氧化能力更加有益。

关键词: 铁源; 抗氧化能力; 肠道健康; 肠道菌群; 大口黑鲈

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铁是鱼类生长所必需的微量元素, 参与体内氧的转运[1]。一般来说, 养殖鱼类所需的铁都要由饲料提供。饲料中铁的含量可以影响鱼类的生长、消化吸收和健康等[2, 3]。研究发现, 铁缺乏与铁过量均会损伤草鱼(*Ctenopharyngodon idella*)鳃的免疫功能[4]。适宜的铁水平可以改善大口黑鲈(*Micropterus salmoides*)的抗氧化能力、免疫力和生长指标等[5, 6]。

无机盐铁(例如: 硫酸亚铁)和有机螯合铁(例如蛋氨酸铁、甘氨酸铁和复合氨基酸螯合铁等)是市场上主要的饲料铁补充剂, 相较于无机盐铁, 有机螯合铁被普遍认为其具有生物学效价高、抗干扰能力强和更容易消化吸收等优点[7]。研究发现黄颡鱼(*Pelteobagrus fulvidraco*)饲料中不同形式的铁源显著影响其铁代谢以及稳态[8]。富马酸亚铁相较于硫酸亚铁可提高鲤(*Cyprinus carpio*)的生长性能和抗病力[9]。在草鱼中也发现蛋氨酸螯合铁和富马酸亚铁相较于硫酸亚铁, 更有利于提高草鱼的健康状

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况与生长性能[4, 10]。

另外, 铁对鱼类的肠道菌群组成也展现出重要的作用[11]。鱼类的肠道菌群具有辅助消化、控制炎症、抵御外来微生物入侵等作用[12]。在大口黑鲈中发现, 饲料中适宜的铁水平有助于增加微生物的多样性[13], 此外, 饲料中铁源的形式同样影响肠道微生物菌落, 相较于硫酸亚铁, 珍珠龙胆石斑鱼(*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂)摄食有机螯合铁饲料时, 其肠道免疫与消化能力得到改善[14]。

大口黑鲈俗称加州鲈, 是我国淡水肉食性鱼类养殖中产量最高的鱼类, 根据《2023中国渔业统计年鉴》显示, 其产量超过80万吨。但大口黑鲈规模化养殖中的病害防控与品质下降问题引起了广泛关注。国内外关于矿物元素(如锌、铜等)添加水平对大口黑鲈肠道健康的研究较多[15—17], 但是有关铁对大口黑鲈肠道影响的研究还较缺乏, 我们前期研究了饲料中铁水平对大口黑鲈生长性能和健康状况的影响[13]。本研究拟探明饲料中不同铁源对大口黑鲈肠道健康的影响, 为大口黑鲈的健康养殖提升提供营养学理论基础。

1　材料与方法

1.1　实验饲料

根据前期确定的适宜铁水平[13], 以硫酸亚铁、蛋氨酸铁、甘氨酸铁和复合氨基酸螯合铁为铁源配制4种等脂等氮的实验饲料(具体配方和营养成分见表 1), 简称$FeSO_4$、Fe-Met、Fe-Gly和Fe-CAA。所用铁源均为亚铁化合物, 各自的铁含量分别为30%、13%、17%和10%, 后3种铁源均由湖南德邦生物科技有限公司提供。饲料原料粉碎后过60目网筛, 混匀后用膨化制粒机制成直径2.5 mm左右的膨化饲料, 双层塑料袋包装并封口, −20℃冰箱保存备用。

1.2　试验用鱼及饲养管理

试验鱼购自武汉得民水产养殖公司, 暂养期间每天8:00和15:00以试验饲料混合物饱食投喂。暂养7d后, 对试验鱼饥饿24h, 并挑选健康, 体格健壮, 规格一致的大口黑鲈随机分入12个圆形养殖缸(300 L水体)中, 每个缸30尾, 针对4种不同铁源的饲料设置4个处理组, 每个处理3个重复, 进行为期70d的养殖试验。试验期间投喂同暂养, 水温(28±1.5)℃; 溶氧>5 mg/L; pH在7.7左右; 硝态氮含量<0.15 mg/L; 铵态氮含量<0.5 mg/L; 自然光照; 水流速: 1.0 L/min; 每天定时进行投喂, 并做好残饵收集与粪便清理的工作。

1.3　样品采集

在养殖试验结束后, 将鱼禁食24h。在每缸中随机抽取5尾鱼, 用稀释的三卡因甲磺酸盐(MS222, 75 mg/L, Aladdin, China)麻醉后, 取其肠道样品, 在液氮中快速冷冻后并储存在−80℃冰箱中, 用于后续的RNA提取与酶活检测及肠道微生物分析等工作。

1.4　样品分析

肠道抗氧化能力测定　肠道抗氧化指标包括超氧化物歧化酶(SOD)、丙二醛(MDA)、谷胱甘肽过氧化物酶(GSH-Px)、总抗氧化能力(T-AOC)及过氧化氢酶(CAT), 均使用建成生物工程研究所(中国南京)生产的试剂盒进行测定。

基因表达测定　使用TRIzol™试剂(TaKaRa, 中国大连)从肠道组织中提取总RNA, TaKaRa的逆转录试剂盒(PrimeScript™ Reagent Kit with gDNA Eraser)将总RNA逆转录为cDNA, 并于–20℃保存备用。通过NCBI数据库获取序列信息, 并使用Primer Premier 5.0软件设计特异性引物(表 2)。qPCR使用Unique Aptamer™ qPCR SYBR® GREEN Master Mix试剂盒在罗氏荧光定量仪上进行检测分析。以*β-actin*为内参, 用$2^{-\Delta\Delta Ct}$的方法计算基因相对表达量。

肠道菌群分析　先利用DNA试剂盒(E.Z.N.A.® soil试剂盒(Omega Bio-tek Norcross, GA, U.S.)提取总DNA。以F(5′-ACTCCTACGGGAGGCAGCAG-3′)和R(5′-GGACTACHVGGGTWTCTAAT-3′)为引物, 对细菌16S rRNA基因的高变区V3—V4进行扩增, 并通过PCR程序获得扩增产物。纯化后, 扩增产物由上海美吉生物医药科技有限公司的Illumina MiSeq PE300平台(Illumina, San Diego, USA)测序。

利用Uprase软件(版本号11)对97%相似水平下的OTU进行聚类, 并去除嵌合体; 选择置信度阈值为0.7, 利用RDP Classifier (版本号2.13)对OTU进行分类学分析并与SLIVA 数据库(版本号138)进行比对。并用Mother (版本号1.30.2)对数据进行alpha多样性分析, 并采用Welch's *t*检验判断组间的差距。然后采用LEFSe差异判别分析, 利用LDA值来衡量物种对差异大小的影响。最后, 通过Tax4Fun (版本号0.3.1)将基于Silva数据库的16S分类谱系转化为KEGG数据库中原核生物的分类谱系, 并对16S RNA基因序列进行KEGG 1—2级功能注释。

1.5　数据统计与分析

文中数据表示为平均值±标准差。基因表达与酶活性测定的数据显著性分析采用SPSS 26.0软件

进行。先对数据进行方差齐性检验, 再通过单因素方差分析和邓肯多重检验来评估不同组别之间的差异。在$P<0.05$时则认为差异显著, 并利用Spearman相关系数来判断酶活与微生物物种之间的相关性, 当$|R|\geqslant 0.8$且$P<0.05$时认为有相关性。

肠道菌群分析中采用到的Welch's *t*检验、Wilcoxon秩和检验以及显著性检验等则利用美吉生物云工具进行分析。

2 结果

2.1 不同铁源对大口黑鲈肠道抗氧化能力的影响

如表 3所示, 不同铁源饲料处理组间的肠道

表 1 不同铁源饲料配方和营养成分

Tab. 1 Different iron-source feed formulations and nutrients (%)

成分Ingredient (%)	组别Group			
	$FeSO_4$	Fe-Met	Fe-Gly	Fe-CAA
鱼粉Fish meal	14.00	14.00	14.00	14.00
酪蛋白Casein	34.70	34.70	34.70	34.70
豆粕Soybean meal	13.00	13.00	13.00	13.00
面粉Wheat flour	12.00	12.00	12.00	12.00
磷酸二氢钙Calcium biphosphate	1.50	1.50	1.50	1.50
矿物质预混料Mineral premix[1]	0.50	0.50	0.50	0.50
氯化钠Sodium chloride	0.25	0.25	0.25	0.25
维生素预混料Vitamin premix[2]	0.50	0.50	0.50	0.50
鱼油+豆油Soy oil: Fish oil (1∶1)	9.00	9.00	9.00	9.00
氯化胆碱Choline chloride (50%)	0.50	0.50	0.50	0.50
乙氧基喹啉Ethoxyquinoline (30%)	0.05	0.05	0.05	0.05
微晶纤维素Microcrystalline cellulose	11.20	11.20	11.20	11.20
羧甲基纤维Carboxymethylcellulose	2.50	2.50	2.50	2.50
防霉剂Mildew inhibitor	0.10	0.10	0.10	0.10
硫酸亚铁Ferrous sulfate (30%)	0.003			
蛋氨酸铁Ferrous methionine chelate (13%)		0.005		
甘氨酸螯合铁Ferrous glycine chelate (17%)			0.004	
复合氨基酸螯合铁Ferrous compound amino acids chelate[3] (10%)				0.006
复合氨基酸Compound amino acids[4]	0.017	0.015	0.016	0.014
营养成分Proximate composition				
水分Moisture (%)	7.47	6.77	6.28	5.76
粗蛋白Crude protein (%)	45.78	45.80	46.44	46.70
粗脂肪Crude lipid (%)	15.33	16.31	14.92	15.58
粗灰分Crude ash (%)	6.20	6.21	6.18	6.16
铁含量Fe content (mg/kg)	80.77	84.59	83.73	79.67

注: [1]每千克矿物质预混料含有: 五水硫酸铜(Ⅱ), 4 g; 一水硫酸锌, 28.57 g; 一水硫酸锰(Ⅱ), 37.5 g; 亚硒酸钠(1%硒), 8 g; 一水硫酸镁, 375 g; 氯化钴(1%钴), 20 g; 碘酸钙(1%), 20 g; 沸石粉, 506.93 g; [2]每千克维生素预混料含有: 维生素 A, 3000000 IU; 维生素 D_3, 1000000 IU; 维生素 E, 64000 mg; 维生素 K_3, 4255 mg; 维生素 B_1, 6061 mg; 维生素 B_2, 6122 mg; 泛酸钙, 20408 mg; 烟酰胺, 30303 mg; 维生素 B_6, 6250 mg; 生物素, 20000 mg; 叶酸, 2041 mg; 维生素 B_{12}, 2000 mg; 维生素C磷酸酯(35%), 228571 mg; 肌醇, 32653 mg; [3]复合氨基酸螯合铁中的氨基酸成分与复合氨基酸相同; [4]复合氨基酸组成成分: 天冬氨酸(Asp), 5.86%; 苏氨酸(Thr), 3.13%; 丝氨酸(Ser), 1.89%; 谷氨酸 (Glu), 7.84%; 甘氨酸(Gly), 4.05%; 丙氨酸(Ala), 5.91%; 胱氨酸(Cys), 0.27%; 缬氨酸(Val), 4.09%; 蛋氨酸(Met), 1. 68%; 异亮氨酸(Ile), 3.07%; 亮氨酸(Leu), 5.67%; 酪氨酸(Tyr), 2.22%; 苯丙氨酸(Phe), 3.46%; 组氨酸(His), 1.37%; 赖氨酸(Lys), 2.97%; 精氨酸(Arg), 4.27%; 脯氨酸(Pro), 2.90%; 色氨酸 (Trp), 1.00%; 氨基酸总量, 61.65%

Note: [1] Per kilogram of mineral premix containing: copper (Ⅱ) sulfate pentahydrate, 4 g; zinc sulfate monohydrate, 28.57 g; manganese (Ⅱ) sulfate monohydrate, 37.5 g; sodium selenite (1% Se), 8 g; magnesium sulfate monohydrate, 375 g; cobalt chloride (1% Co), 20 g; calcium iodate (1%), 20 g; zeolite powder, 506.93 g; [2]Per kilogram of vitamin premix containing: vitamin A, 3000000 IU; vitamin D_3, 1000000 IU; vitamin E, 64000 mg; vitamin K_3, 4255 mg; vitamin B_1, 6061 mg; vitamin B_2, 6122 mg; calcium pantothenate, 20408 mg; nicotinamide, 30303 mg; vitamin B_6, 6250 mg; biotin, 20000 mg; folic acid, 2041 mg; vitamin B_{12}, 2000 mg; L-ascorbate-2-monophosphate (35%), 228571 mg; inositol, 32653 mg; [3]The ferrous compound amino acids chelate has the same amino acid composition as that of the compound amino acids; [4]Compound amino acids composition: aspartic acid (Asp), 5.86%; Threonine (Thr), 3.13%; Serine (Ser), 1.89%; Glutamic acid (Glu), 7.84%; Glycine (Gly), 4.05%; Alanine (Ala), 5.91%; Cystine (Cys), 0.27%; Valine (Val), 4.09%; Methionine (Met), 1.68%; Isoleucine (Ile), 3.07%; Leucine (Leu), 5.67%; Tyrosine (Tyr), 2.22%; Phenylalanine (Phe), 3.46%; Histidine (His), 1.37%; Lysine (Lys), 2.97%; Arginine (Arg), 4.27%; Proline (Pro), 2.90%; Tryptophan (Trp), 1.00%; Total amino acids, 61.65%

SOD和GSH-Px活性差异不显著(P>0.05)。相较于$FeSO_4$组、Fe-Met组和复合Fe-CAA组肠道的T-AOC活性显著升高(P<0.05)。而Fe-Met组CAT活性显著高于其他组(P<0.05), $FeSO_4$组MDA含量显著高于其他组(P<0.05)。

2.2　不同铁源对大口黑鲈肠道健康相关基因表达的影响

如图 1所示, 在肠道健康相关基因表达中, $FeSO_4$组的*IL-8*和*Occludin*基因的相对表达量显著高于其余3组(P<0.05); Fe-Gly和Fe-CAA组的*ZO-1*和*Claudin-1*基因的相对表达量则显著高于其他两组(P<0.05)。而不同铁源饲料之间的*IL-10*、*IL-6*及*IL-15*基因的相对表达差异不显著。

2.3　不同铁源对大口黑鲈肠道菌群多样性的影响

根据前面指标的结果, 本文选择$FeSO_4$组和Fe-CAA组的肠道样品进一步进行菌群分析。焦磷酸测序后, 对6个样本进行抽平, 按照其中的最小样本序列值统一为40084个序列。使用97%的序列相似性, 得到OTU个数为230。Shannon曲线和Sobs稀释曲线(图 2)随着测序深度趋于平台的饱和期, 说明不同样品中的微生物多样性和总OTU已经不随测序深度变化而产生变化。

Sobs、Chao和Ace都是用来评估大口黑鲈肠道菌群丰富度的指标(图 3)。$FeSO_4$组的菌群丰富度显著高于Fe-CAA组(Sobs与Chao, P<0.001; Ace, P<0.01)。此外, alpha多样性显示出较高的物种覆盖指数, 表明测序深度足以覆盖样本的物种丰富度。Simpson与Shannon作为评估大口黑鲈肠道菌群多样性的指标, 也受到铁源的显著影响(P<0.001)。通过加权UniFrac计算的PCoA结果显示, 两组分离明显。对其进行进一步ANOSIM分析发现“Between”所代表的两样品组间差距是高于两组样品的组内差距的。

表 2　荧光定量引物序列

Tab. 2　Sequence of fluorescence quantitative primers

基因 Gene	引物序列 Primer sequence (5′—3′)	登录号 GenBank No.
IL-10	CCACCAGAATGACTCCTCGG TGGTTGTTGCACATGGGACT	XM_038696252
IL-6	CGCGCAATTTGCCGATGATA CGTTGTTGCTGGTTGCATGA	XM_038732985
IL-15	GTATGCTGCTTCTGTGCCTGG AGCGTCAGATTTCTCAATGGTGT	XM_038693988
IL-8	CGTTGAACAGACTGGGAGAGATG AGTGGGATGGCTTCATTATCTTGT	XM_038704088
Occludin	GATATGGTGGCAGCTACGGT TCCTACTGCGGACAGTGTTG	XM_038715419
ZO-1	ATCTCAGCAGGGATTCGACG CTTTTGCGGTGGCGTTGG	XM_038701018
Claudin-1	CCAGGGAAGGGGAGCAATG GCTCTTTGAACCAGTGCGAC	XM_038713307
β-actin	AAAGGGAAATCGTGCGTGAC AAGGAAGGCTGGAAGAGGG	XM_038695351

表 3　不同铁源对大口黑鲈肠道抗氧化酶活性的影响

Tab. 3　Effects of different iron sources on intestinal antioxidant enzyme activities of largemouth bass

指标Item	组别 Group			
	$FeSO_4$	Fe-Met	Fe-Gly	Fe-CAA
超氧化物歧化酶 SOD (U/mg prot)	6.19±0.84	6.21±0.45	6.55±0.52	7.32±0.84
谷胱甘肽过氧化物酶 GSH-Px (U/mg prot)	10.70±0.80	10.55±0.64	11.16±1.10	11.53±1.12
总抗氧化能力T-AOC (mmol/g prot)	0.21±0.02^{b}	0.25±0.01^{a}	0.23±0.02^{ab}	0.25±0.01^{a}
过氧化氢酶CAT (U/mg prot)	4.64±0.35^{b}	5.51±0.02^{a}	4.62±0.38^{b}	4.83±0.19^{b}
丙二醛MDA (nmol/mg prot)	2.10±0.14^{a}	1.96±0.17^{b}	1.71±0.14^{b}	1.80±0.03^{b}

注: 所有指标数据均为3个重复(n=3)的平均值, 同一指标数据中不同的上标字母表示各处理组间有显著性差异(P<0.05)

Note: All data are means of three replicates (n=3). Different superscript letters in the same index data indicate significant differences among the treatment groups (P<0.05)

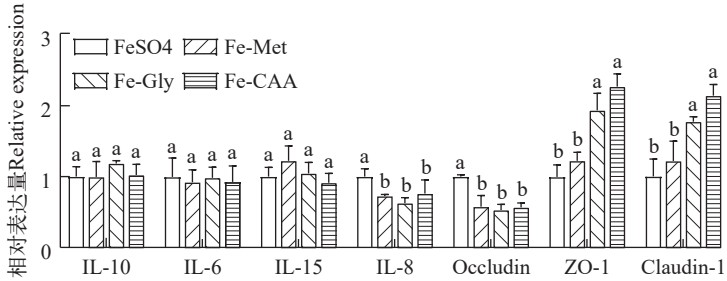

图 1　不同铁源对大口黑鲈肠道炎症因子(*IL-10*、*IL-6*、*IL-15*和*IL-8*)和紧密连接蛋白(*Occludin*、*ZO-1*和*Claudin-1*)基因表达的影响

Fig. 1　Effects of different iron sources on intestinal inflammatory factors (*IL-10*, *IL-6*, *IL-15*, and *IL-8*) and tight junction proteins (*Occludin*, *ZO-1*, and *Claudin-1*) gene expression in largemouth bass

所有指标数据均为3个重复(n=3)的平均值; 同一指标数据中不同的上标字母表示各处理组间有显著性差异(P<0.05)

All data are means of three replicates (n=3); Different superscript letters in the same index data indicate significant differences among the treatment groups (P<0.05)

2.4 不同铁源对大口黑鲈肠道菌群组成的影响

如图 4所示, 在门水平上, 不同铁源处理组的优势门是变形菌门($FeSO_4$: 43.37%, Fe-CAA: 74.10%)、厚壁菌门($FeSO_4$: 46.48%, Fe-CAA: 6.55%)和梭杆菌门($FeSO_4$: 8.58%, Fe-CAA: 19.13%)。在属水平上, 丰度比最高的6个属是*Plesiomonas* ($FeSO_4$: 9.47%, Fe-CAA: 36.10%)、*Enterobacter* ($FeSO_4$: 12.59%, Fe-CAA: 29.11%)、*Cetobacterium* ($FeSO_4$: 8.58%, Fe-CAA: 19.13%)、*Lactococcus* ($FeSO_4$: 20.20%, Fe-CAA: 2.88%)、*Aeromonas* ($FeSO_4$: 8.30%, Fe-CAA: 5.84%)和*Staphylococcus* ($FeSO_4$: 8.30%, Fe-CAA: 5.84%)。而有关大口黑鲈肠道菌群属水平的相对丰度与肠道抗氧化酶活性的相关性检验(图 5)表明, 肠道MDA活性与*Escherichia-Shigella* (r= –0.899)、*Enterobacter*(r=–0.886)、*Pantoea*(r=0.886)、*Chryseobacterium*(r=0.886)、*Comamonas*(r=0.829)、*Acinetobacter* (r=0.829)、*Pseudomonas* (r=0.829)、*Lactococcus* (r=0.886)和*Weissella* (r=0.943)具有强烈的相关性。肠道T-AOC活性与*Enterobacter* (r=0.812)、*Plesiomonas* (r=0.928)、*Raoultella* (r=0.928)、*Achromobacter*(r=–0.986)、*Pedobacter*(r=–0.928)、*Streptococcus* (r=–0.941)、*Comamonas* (r=–0.841)、*Staphylococcus*(r=–0.928)、*Aerococcus*(r=–0.928)、*Macrococcus*(r=–0.893)、*Acinetobacter*(r=–0.841)、*Pseudomonas* (r= –0.841)和*Lactococcus* (r= –0.812)也显示出强烈的相关性。此外, 肠道SOD活性与*Achromobacter* (r= –0.829)有强烈的负相关, 肠道GSH活性与*Streptococcus* (r= –0.812)也显示出强烈负相关。

LEfSe多级物种差异判别分析则展现了两种不同铁源饲料处理组在不同层级上物种组成的差异(图 6)。$FeSO_4$组主要富集的是变形菌门, 而Fe-CAA组主要富集的是厚壁菌门。

2.5 不同铁源对大口黑鲈肠道功能的影响

KEGG1级结果显示, 两处理组微生物主要功能预测聚集在“新陈代谢”与“环境信息处理”上。KEGG2级结果显示, 两处理组微生物的主要功能最可能是“碳水化合物代谢”“跨膜运输”和“氨基酸代谢”(图 7)。

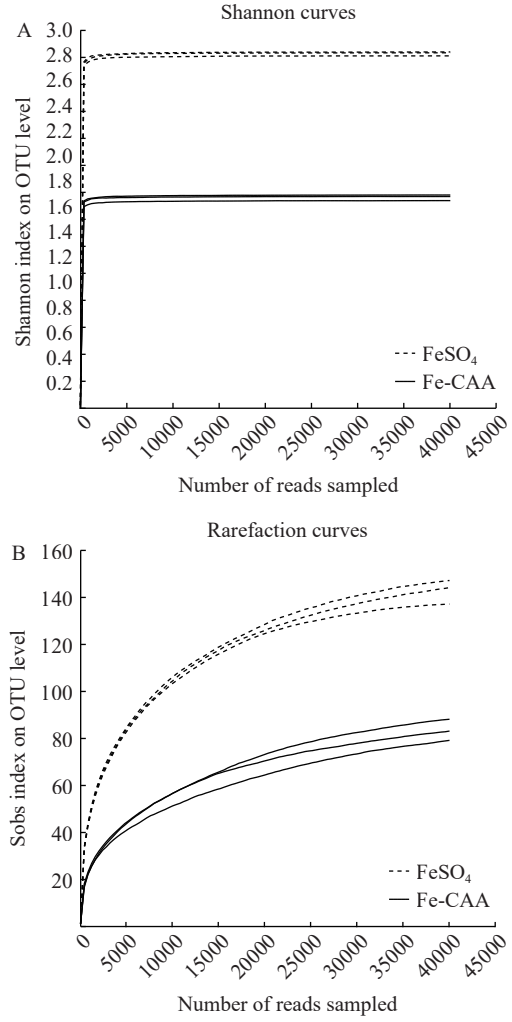

图 2 在不同铁源处理下大口黑鲈肠道微生物群OTU水平下的Shannon曲线(A)和Sobs稀释曲线(B)

Fig. 2 Shannon curve (A) and Sobs dilution curve (B) of gut microbiota of largbass at OTU level under different iron source treatments

横轴为有效序列数, 纵轴为观察到的操作分类单元的数量; Shannon指数用于评估肠道微生物的多样性, 而Sobs指数则是用于评估肠道微生物物种数量(丰度)

The horizontal axis is the number of effective sequences and the vertical axis is the number of operational taxa observed; The Shannon index is used to assess the diversity of gut microbes, while the Sobs index is used to assess the number of gut microbial species (abundance)

3 讨论

3.1 不同铁源对大口黑鲈抗氧化能力的影响

MDA作为油脂氧化酸败后产生的一种有害物质[18], 常用来作为评判机体氧化损伤的指标。过多的自由基会攻击生物膜, 生成脂质过氧化物, 并最终分解成MDA, 而抗氧化物酶(T-AOC、SOD、CAT和GSH-P等)可以清除机体类活性氧自由基[19]。本研究发现Fe-CAA和Fe-Met组大口黑鲈肠道的T-AOC含量提高, 能够提升机体的抗氧化能力。此外, Fe-Met能够显著提升肠道的CAT含量。然而不同铁源并没有对SOD和GSH-Px含量产生显著影响。有研究发现, 饲料中添加适宜的甘氨酸铁可以提高肉鸡和仔猪的免疫力[20, 21], 这与本研究结果相似, 本研究中以甘氨酸铁和复合氨基酸螯合铁作为铁源时MDA的活性相对较低, 说明二者作为铁源可以降

低肠道氧化损伤。这些研究结果表明不同形式的铁源对大口黑鲈的抗氧化能力的影响不同, 其可能原因是大口黑鲈对于不同铁源的吸收利用能力及耐受性不同[22]。

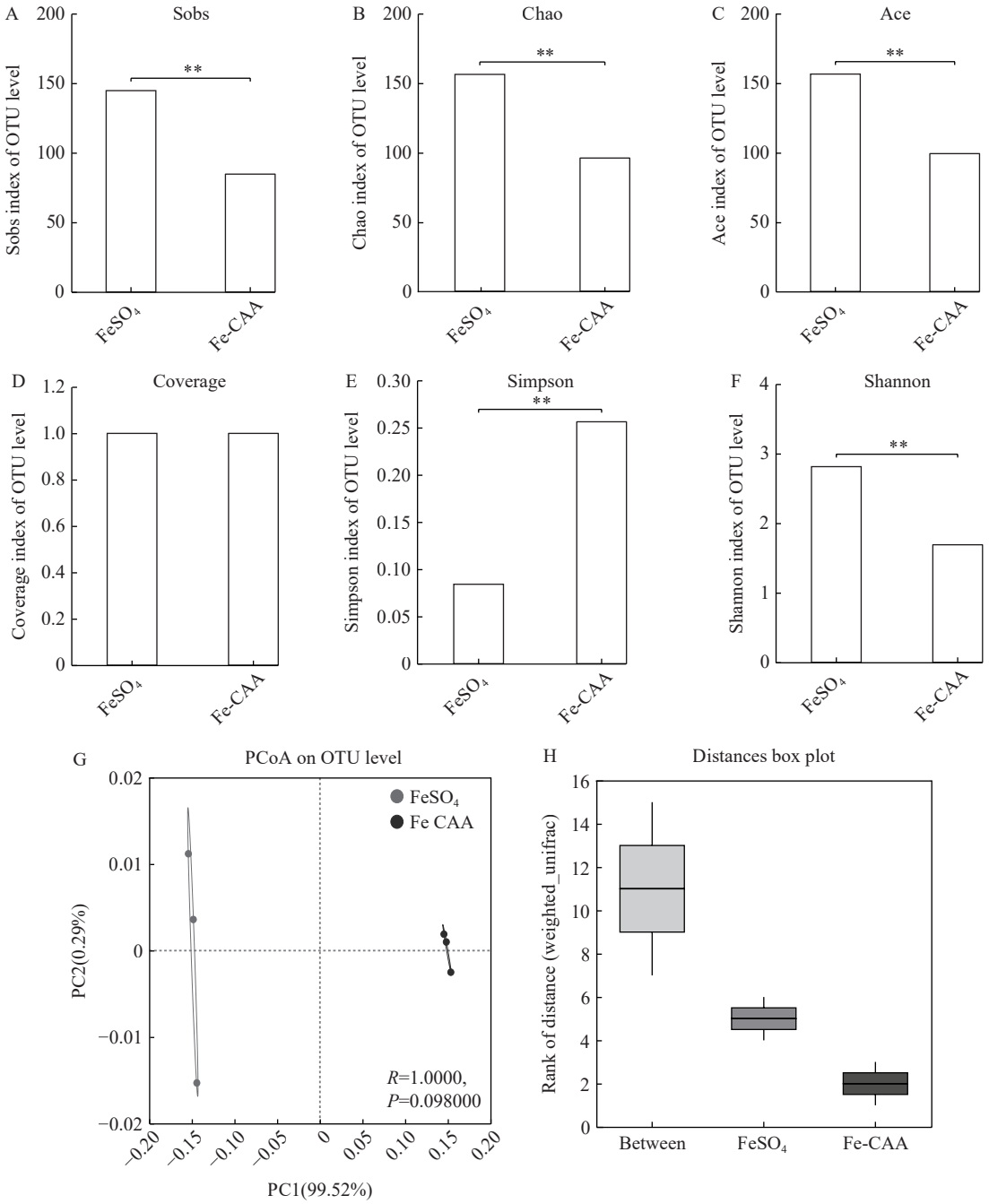

图 3　在不同铁源处理下大口黑鲈肠道菌群的Alpha多样性和Beta多样性分析

Fig. 3　Alpha and Beta diversity analysis of intestinal flora in largemouth bass under different iron sources

Sobs (A)、Chao (B)和Ace (C)为评估肠道菌群丰富度的指标; Coverage (D)为评估群落覆盖率的指标。而Simpson (E)和Shannon (F)为评估肠道菌群多样性的指标; A—F指标均采用Welch's *t*检验进行显著性分析, 对差异显著的两组进行标记(*P≤0.05, **P≤0.01); G. 肠道微生物群落基于加权UniFrac的主坐标分析; H. 微生物群落不同铁源组的相似性分析; "Between"箱子代表组间差异, 而其他箱子则代表组内差异

Sobs (A), Chao (B), and Ace (C) are used to evaluate the abundance of intestinal flora; Coverage (D) is an indicator to evaluate community coverage. Simpson (E) and Shannon (F) are used to evaluate the diversity of intestinal flora. Welch's *t* test is used for significance analysis of A—F indexes, and the two groups with significant differences are labeled (*P≤0.05, **P≤0.01); G. Principal coordinate analysis of gut microbial community based on weighted UniFrac; H. Similarity analysis of different iron source groups in microbial communities; The "Between" boxes represent inter-group differences, while the other boxes represent intra-group differences

3.2 不同铁源对大口黑鲈肠道健康相关基因表达的影响

肠道是鱼类重要的免疫屏障, 在抵御疾病方面具有重要的作用。鱼类机体中的细胞因子通过特殊的方式调节机体的免疫能力, 从而维持机体的稳定[23], 如*IL-10*对鱼B细胞的分化、增殖和吞噬具有调控作用[24]。研究表明抗炎因子表达升高与促炎因子表达降低均有利于提升机体免疫, 从而控制炎症反应[25]。抗炎因子*IL-10*和促炎因子*IL-6*、*IL-15*、*IL-8*均是对肠道免疫反应有一定调控作用的细胞因子, 本研究发现, Fe-Met、Fe-Gly和Fe-CAA相较于$FeSO_4$这种铁源添加形式, 对促炎因子*IL-8*的表达具有一定的抑制作用, 有利于提升肠道的免疫能力, 而抗炎因子*IL-10*、促炎因子*IL-6*与*IL-15*则没有受到铁源的显著影响。此外, *Occludin* (闭锁蛋白)、*ZO-1* (闭锁小带蛋白-1)和*Claudin-1* (跨膜蛋白-1)作为上皮及内皮细胞中的重要调节蛋白, 能够对上皮以及内皮屏障的功能产生重要的影响[26]。本研究发现Fe-Gly和Fe-CAA组显著提升*ZO-1*与*Claudin-1*的表达水平, 有利于大口黑鲈的肠道健康, 这与之前不同铁源对草鱼的健康研究较为一致[10]。然而实验中有机铁源的添加却降低了*Occludin*的表达, 增大了肠道的通透性, 这可能是因为$FeSO_4$作为铁源与有机铁作为铁源相比, 肠道菌落结构受到了改变, 进而影响到了肠道免疫系统的发育, 同时影响了基因的表达[27]。

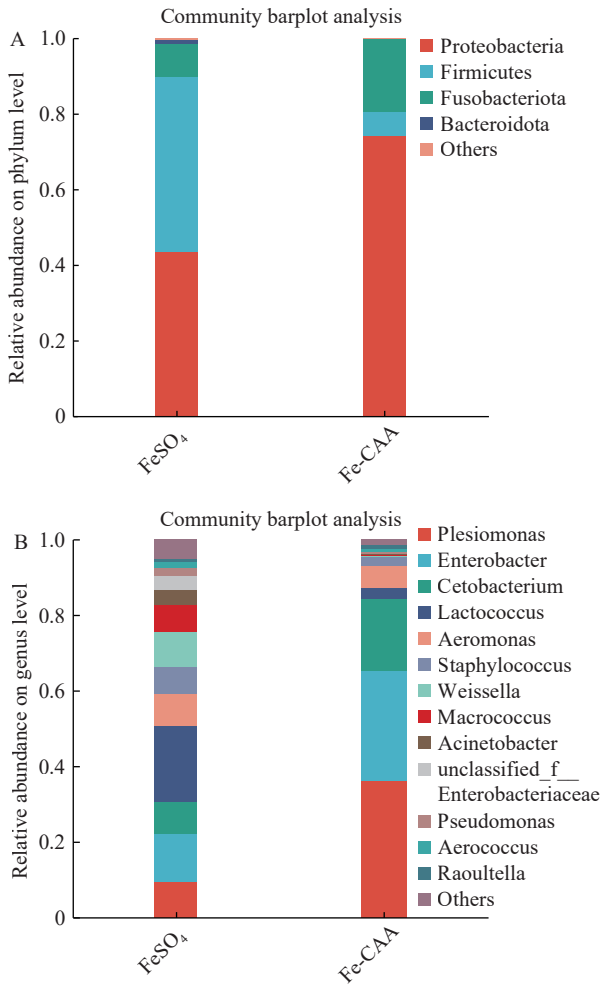

图 4 不同铁源饲料处理组的肠道微生物组成

Fig. 4 Intestinal microbial composition in different iron-source feed treatment groups

具体为饲喂70d后大口黑鲈肠道微生物在门(A)和属(B)水平上的组成。其中“others”为丰度<1%的类群

Specifically, the composition of intestinal microorganisms of largemouth bass at phylum (A) and genus (B) level after 70d of feeding. Among them, “others” is a group whose abundance is<1%

3.3 不同铁源对大口黑鲈肠道菌群的影响

肠道菌群与蛋白质代谢、淀粉消化和维生素合成等都息息相关, 对动物机体的代谢、免疫等均有着重要的作用[28]。目前肠道菌群逐渐受到研究者的关注[29]。本研究将$FeSO_4$和Fe-CAA组的肠道微生物通过16S扩增子测序并比较Shannon和Simpson指数后发现, Fe-CAA的添加形式会降低大口黑鲈肠道微生物的多样性, 且 PCoA结果也显示两个组之间分离明显, 但是多样性差距不显著。

对两组的菌群进行比较分析发现, 在门水平上, 大口黑鲈肠道中的优势菌群为变形菌门、厚壁菌门和梭杆菌门, 这与已有的研究较为一致[30]。变形菌群的丰度变化往往是肠道菌群失调的表现, 而之前对大口黑鲈不同发育阶段肠道菌群的研究则指出变形菌门的相对丰度能够维持在80%以上[31]。在本实验中, Fe-CAA组中的菌落结构与其类似, 而$FeSO_4$组的变形菌群比例则相对偏低, 本文推测Fe-CAA可能通过调节变形菌群的相对丰度来促进肠道菌群达到相对平衡的状态。在属水平上, 群落组成图和LEfSe分析表明, $FeSO_4$组*Lactococcus*在大口黑鲈肠道中相对丰度最高, 而Fe-CAA组中的*Plesiomonas*、*Enterobacter*和*Cetobacterium*则相对具有较高的丰度, 同时两处理组在*Weissella*等多个属中都存在差异(LDA>4)。有研究表明, 适当的乳球菌属(*Lactococcus*)可以促进鳜的生长, 而过量则会对生长性能、肝肠健康与微生物的多样性产生有害影响[32]。而有关邻单胞菌属(*Plesiomonas*)的研究表明, 其可能会导致鱼类的败血症[33], 但也有研究表明其可以在一定程度上刺激免疫应答[34], 推测邻单胞菌属处在适当水平有利于刺激免疫反应, 而菌落失衡则可能会引发疾病。在本研究中, 复合氨基酸螯合铁组展现出较低的MDA水平也可能是与邻

单胞菌属有关。此外, 还有研究表明*Plesiomonas*可以显著增加变形菌门细菌的丰度并改变其他菌门的相对丰度从而引起肠道菌群物种多样性与丰度的显著变化[35], 这也与本实验结果一致。另外, 鲸杆菌属(*Cetobacterium*)也被发现与大口黑鲈的肠道健康与免疫相关, 其不仅是一种可以潜在抵御诺卡氏菌的益生菌, 而且可以提升鱼体的抗氧化能力[36]。总体比较而言, Fe-CAA组虽然肠道菌落多样性更低, 但是菌落结构相对更好, 而$FeSO_4$组则可能是出现了菌落失衡的情况, 这可能是由于乳球菌属成为了优势种属, 从而改变了肠道菌落结构, 破坏了菌落平衡。同时, Spearman检验结果显示, MDA水平与*Lactococcus*和*Weissella*呈正相关, 与*Enterobacter*呈负相关, 而T-AOC水平则与*Plesiomonas*和*Enterobacter*呈正相关, 与*Lactococcus*呈负相关。这些结果体现出肠道菌群与肠道抗氧化能力是息息相关的, 且$FeSO_4$组的优势菌属会升高MDA含量并降低T-AOC, 而Fe-CAA组则相反, 在降低MDA的过程中提高了肠道的抗氧化能力。这一结果为我们通过营养途径来调控水产品抗氧化能力提供了新的思路。

最后, 利用Tax4Fun微生物群落功能预测分析, 发现两种不同铁源的处理组最丰富的肠道菌群功能均为“碳水化合物代谢”“跨膜运输”和“氨基酸代谢”, 这与另一项对大口黑鲈肠道菌群的研究十分一致[37]。此外, 结果显示, 微生物群落功能在两种不同铁源的处理组之间无显著差异, 这可能是由于肠道菌群的功能并不仅仅是受限于由一两种微生物的影响, 而是受到肠道中多种微生物的共同影响[38, 39]。

4　结论

综上所述, 相较于无机铁源, 有机铁源(蛋氨酸铁、甘氨酸铁和复合氨基酸螯合铁)更能够提升大口黑鲈幼鱼的肠道抗氧化能力, 并降低MDA含量, 进而降低肠道损伤, 同时饲料中添加复合氨基酸螯

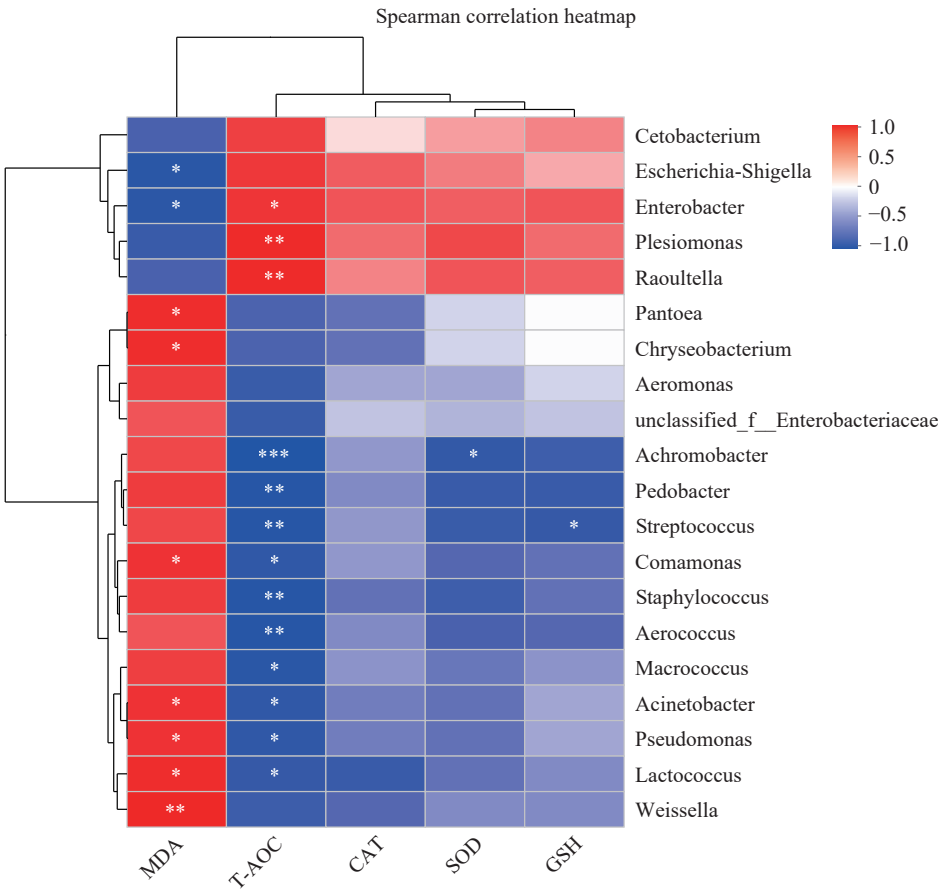

图 5　大口黑鲈肠道菌群属(genus)水平的相对丰度与肠道抗氧化酶活性的相关性检验

Fig. 5　Correlation between the relative abundance of intestinal flora at genus level and intestinal antioxidant enzyme activity of large-mouth bass

斯皮尔曼检验, 其中*P<0.05, **P<0.01, ***P<0.001

Spearman test, where *P<0.05, **P<0.01, ***P<0.001

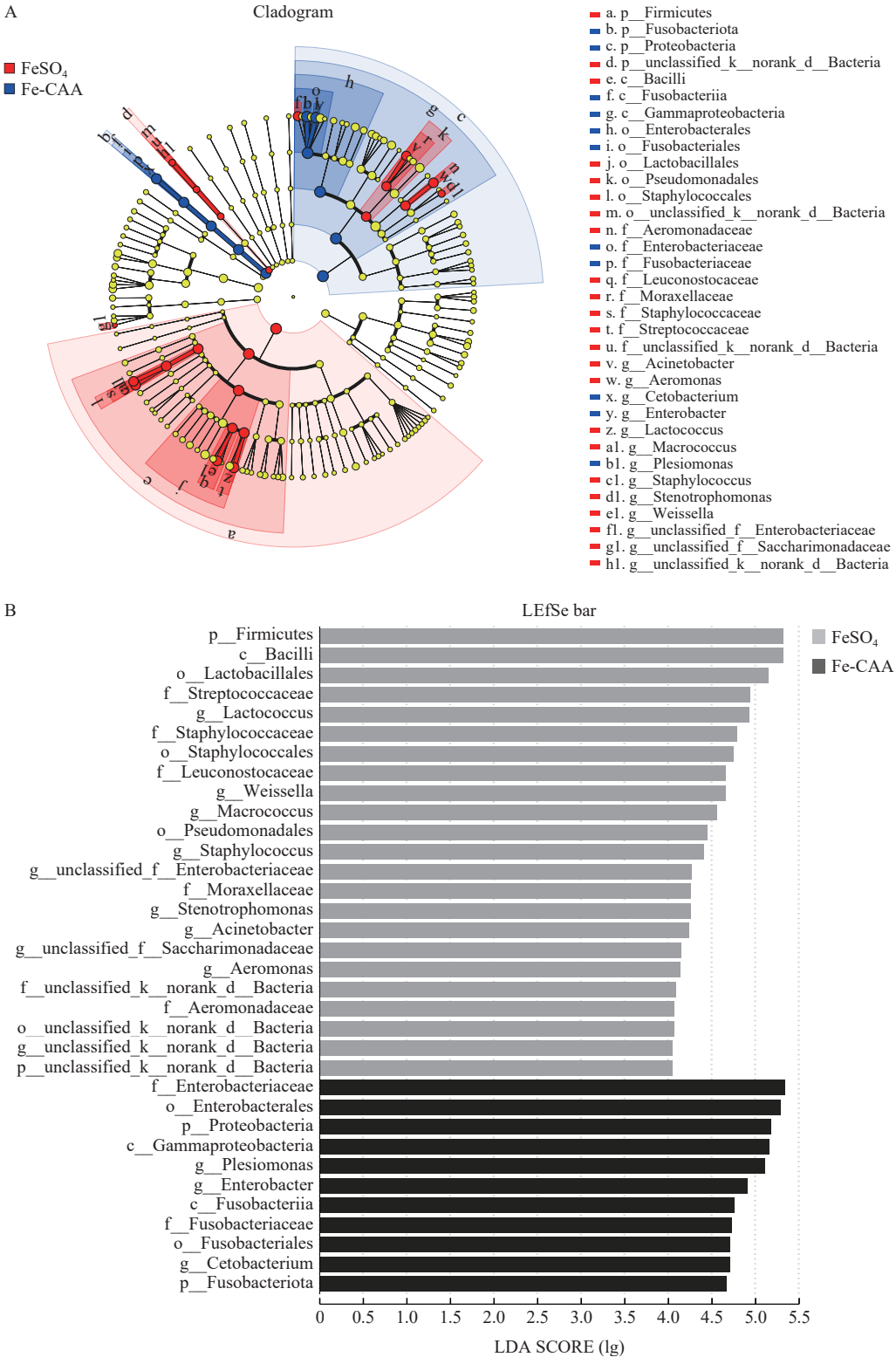

图 6 LEfSe多级物种差异判别分析下不同铁源饲料处理组的物种差异分布

Fig. 6 Species difference distribution of different iron source feed treatment groups under LEfSe multilevel species difference discriminant analysis

A. 分支图显示了LEfSe分析下不同铁源饲料处理组的系统发育分布(分支图由内到外分别代表门、纲、目、科和属五个不同的分类水平); B. LDA评分用来衡量不同铁源饲料处理组的物种对差异效果影响大小(LDA>4.0, P<0.05)

A. The branching diagram shows the phylogenetic distribution of different iron source feed treatment groups under LEfSe analysis (the branching diagram represents five different taxonomic levels of phyla, class, order, family, and genus from the inside out); B. LDA score is used to measure the influence of species on the difference effect of different iron source feed treatment groups (LDA>4.0, P<0.05)

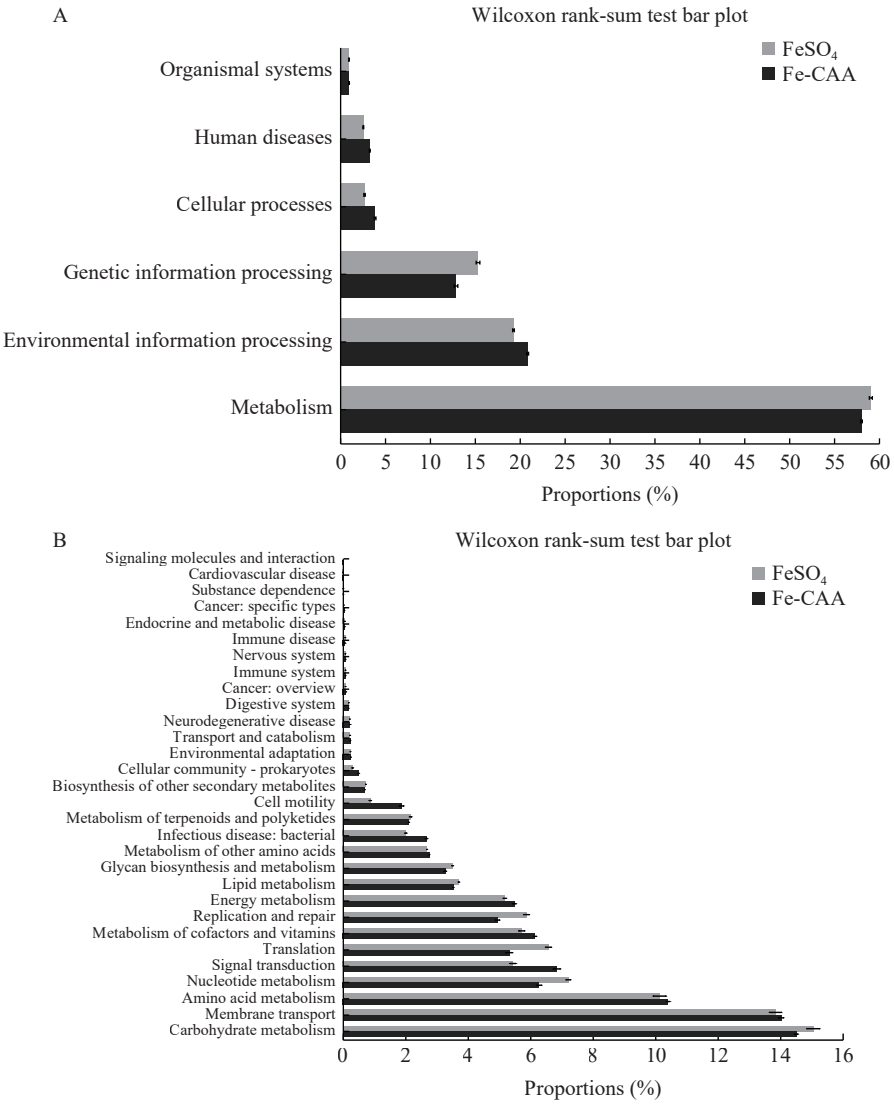

图 7　不同铁源饲料处理的大口黑鲈肠道菌群的Tax4Fun功能预测

Fig. 7　Tax4Fun function prediction of intestinal microflora of largemouth bass treated with different iron source diets

A. KEGG1水平下的不同处理组的菌群功能预测; B. KEGG2水平下的不同处理组的菌群功能预测; 显著性检验采用的是Kruskal-Wallis秩和检验(n=3)

A. Prediction of flora function in different treatment groups at KEGG1 level; B. Prediction of flora function in different treatment groups at KEGG2 level. Significance test is performed by Kruskal-Wallis rank sum test (n=3)

合铁有利于维持大口黑鲈肠道菌群的稳定性, 提高肠道内有益菌群的丰度。

(作者声明本文符合出版伦理要求)

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DIFFERENT DIETARY IRON SOURCES ON INTESTINAL HEALTH OF LARGEMOUTH BASS (*MICROPTERUS SALMOIDES*)

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Abstract: Fish health, as well as disease prevention and control, are the key and tricky sectors in aquaculture. It is of great significance to improve fish immunity and disease resistance through nutritional regulation. Iron is an essential nutrient that regulates growth and immunity in fish. The purpose of this study was to investigate the regulatory effects of ferrous sulfate ($FeSO_4$), methionine chelated iron (Fe-Met), glycine chelated iron (Fe-Gly), and complex amino acid chelated iron (Fe-CAA) as dietary iron source on intestinal health of largemouth bass (*Micropterus salmoides*). Four isonitrogenous and isolipidic diets with appropriate iron level provided by $FeSO_4$, Fe-Met, Fe-Gly, or Fe-CAA were designed. Three replicates were set in each group for a 70-day breeding experiment of young largemouth bass. After the experiment, intestinal antioxidant activities of SOD, CAT, and T-AOC were measured in each group. Additionally, the expression of genes and flora related to intestinal health were analyzed and compared. The results showed that the MDA content of $FeSO_4$ group was significantly higher than that of Fe-Gly and Fe-CAA groups (P<0.05), while the T-AOC activity of $FeSO_4$ group was significantly lower than that of Fe-Met and Fe-CAA groups (P<0.05). The CAT level of Fe-Met group was significantly higher than that of the other three groups. At the gene level, the expressions of *IL-8* and *Occludin* genes were significantly higher in $FeSO_4$ group than those in the other groups (P<0.05), while the expressions of *ZO-1* and *Claudin-1* genes were significantly higher in Fe-Gly and Fe-CAA group than those in $FeSO_4$ and Fe-Met groups (P<0.05). The expressions of *IL-10*, *IL-6*, and *IL-15* genes were not significantly affected by iron sources. Further analysis of intestinal flora showed that the bacterial diversity of Fe-CAA group was significantly lower compared with $FeSO_4$ group (P<0.05). At the phylum level, Firmicutes in Fe-CAA group were significantly down-regulated, while Proteobacteria and Clostriobacteria were significantly up-regulated compared with $FeSO_4$ group (P<0.05). At the genus level, *Plesiomonas*, *Enterobacter*, and *Cetobacterium* in Fe-CAA group were significantly up-regulated, while *Lactococcus* and *Aeromonas* were significantly down-regulated (P<0.05). Correlation analysis of intestinal flora and antioxidant enzymes showed that intestinal MDA activity was strongly correlated with Escherichia-Shigella and Enterobacter. Intestinal T-AOC activity also showed a strong correlation with Plesiomonas and Achromobacter, while SOD activity was strongly negatively correlated with Achromobacter. Additionally, a strong negative correlation was found between intestinal GSH activity and Streptococcus. However, microbial community function prediction analysis showed no significant difference in intestinal microbial community function between the two groups. In conclusion, compared with inorganic iron sources, organic iron sources can improve the intestinal antioxidant capacity of juvenile largemouth bass, reduce the content of MDA, and thus minimize intestinal damage. Meanwhile, the inclusion of iron complex amino acid chelate in the diet is beneficial to maintain the stability of intestinal flora of largemouth bass and improve the abundance of beneficial flora in the intestine.

Key words: Iron source; Antioxidant capacity; Intestinal health; Intestinal flora; *Micropterus salmoides*