

饲料中添加大型海藻对感染GCRV草鱼肠道菌群和免疫的影响

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MACROALGAL SUPPLEMENTED DIET ON GUT MICROBIOTA AND IMMUNITY OF GRASS CARP DURING GCRV INFECTION

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饲料中添加大型海藻对感染GCRV草鱼肠道菌群和免疫的影响

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摘要: 为研究饲料中添加大型海藻对感染呼肠孤病毒(GCRV)的草鱼(*Ctenopharyngodon idella*)肠道菌群和免疫的影响, 在草鱼饲料中分别添加5%的马尾藻(*Sargassum hemiphyllum*)、紫杉状海门冬(*Asparagopsis taxiformis*)、龙须菜(*Gracilaria lemaneiformis*)藻粉, 饲喂草鱼2个月。采用腹腔注射, 构建草鱼GCRV感染模型, 分析宿主肠道微生物群落和免疫响应。结果显示, 饲料中添加大型海藻显著提高了健康草鱼肠道菌群的多样性, 改变了特有微生物数量。有效减缓了GCRV感染1d后的草鱼肠道菌群多样性的降低, 缩小了GCRV感染4—7d草鱼肠道菌群的组间差异, 降低了感染后肠道菌群的紊乱。其中饲料中添加马尾藻和龙须菜组肠道中弧菌属(*Vibrio*)相对丰度显著降低; 饲料中添加马尾藻组肠道中鲸杆菌属(*Cetobacterium*)相对丰度降低, 而拟杆菌属(*Bacteroides*)相对丰度增加。共现网络分析显示, 大型海藻提高了感染GCRV的草鱼肠道菌群现网络的复杂度, 增强肠道菌群的稳定性。蛋白表达结果显示, 感染后1d, 饲料中添加马尾藻组草鱼血清中促炎补体成分C5a含量增加, 而饲料中添加龙须菜组补体成分C5a含量降低。相关性分析表明, 补体蛋白C5、C5a成分及其受体(C5aR)的上调表达与肠道中致病菌群相对丰度显著正相关, 而与碳水化合物和膳食纤维消化菌群负相关。综上, 饲料中添加大型海藻缓解了GCRV感染对草鱼肠道菌群带来的不利影响, 并调节感染草鱼的补体表达, 可为从饲料开发方面防控草鱼出血病提供新思路。

关键词: 草鱼出血病; 大型海藻; GCRV; 肠道微生物; 补体

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草鱼(*Ctenopharyngodon idella*)是我国主要的淡水养殖鱼类之一, 2022年全国总产量近600万吨^[1]。由于在养殖过程中容易爆发草鱼出血病, 常给草鱼养殖业带来巨大经济损失^[2]。草鱼出血病是由草鱼呼肠孤病毒(GCRV)感染引起的病毒性疾病, 发病迅速且死亡率高^[3, 4], 目前主要以疫苗预防为主^[5]。由于肠道微生物与宿主之间存在密切的相互作用^[6, 7], 通过维持肠道微生物的多样性和稳定性, 可增强草鱼对GCRV的免疫能力和缓解疾病带来的影响^[8-10]。补体系统是先天免疫的重要组成部分, 连接先天免疫和适应性免疫^[11]。具有诱发炎症和溶解杀灭体内受感染细胞的功能^[12], 是鱼类抵御病毒感染时的

第一道屏障^[13, 14]。补体系统通过识别不同的抗原并形成不同的C5转化酶, 催化补体C5裂解为C5a分子和C5b分子, C5a与受体C5aR形成炎症调控通路调节炎症反应。研究发现, 草鱼感染GCRV后的炎症反应是C5a结合C5aR形成C5a/C5aR通路, 促进p38蛋白和ERK蛋白磷酸化, 调控促炎因子IL-6和TNF- α 的表达, 调控草鱼感染GCRV后的炎症反应进程^[15, 16]。

大型海藻种类多、分布广、产量大^[17], 含有丰富的藻类多糖、海藻酸盐和藻多酚等天然有效活性物质, 以及丰富的膳食纤维和矿物质^[18], 可调控动物营养物质的代谢和吸收, 被证明具有抗病毒和

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抗氧化活性等功效^[19–21], 目前已经作为天然饲料原料用于水产动物养殖, 以改善鱼类抗病能力^[22]。但具体的作用途径和影响机制不清楚。

本研究以褐藻门中的马尾藻(*Sargassum hemiphyllum*)、红藻门中的紫杉状海门冬(*Asparagopsis taxiformis*)和龙须菜(*Gracilaria lemaneiformis*)为实验材料, 以饲料总重5%的干藻粉分别添加制成实验饲料饲喂草鱼2个月, 腹腔注射GCRV方式构建草鱼GCRV感染模型, 检测草鱼肠道微生物和草鱼血液中C5、C5a和C5aR含量, 研究饲料中添加大型海藻对草鱼感染GCRV肠道菌群和免疫的影响, 为探究大型海藻作为潜在抗病天然活性材料研究提供基础数据。

1 材料与方法

1.1 实验设计

选择体重为(91.50±0.66) g健康草鱼360尾, 进行7d养殖驯化。然后将其随机分成4组, 包括对照组(C), 饲料中添加大型海藻组: 马尾藻组(S)、紫杉状海门冬组(A)、龙须菜组(G)。每组3个生物学重复, 每个重复30尾草鱼。对照组投喂100%基础饲料, 马尾藻组、紫杉海门冬组、龙须菜组则在95%基础饲料配方上分别添加5%对应的大型海藻粉(采自珠江三角洲海域), 将各种原料按照配方称量后进行超微粉碎, 用80目筛进行过滤筛分, 充分混匀, 用膨化饲料机制作成直径2.0 mm的颗粒, 于75℃的温度烘干, 冷却后保存于4℃冷藏箱中备用。测定3种大型海藻的营养成分制作营养成分表1和草鱼饲料营养配比表2。

各组草鱼饲喂2个月后, 从每个养殖桶随机取出3尾草鱼隔离饲养至相同养殖环境中, 作为感染对照组(注射0.5 mL PBS液), 剩余草鱼则进行病毒腹腔注射作为感染组(注射0.5 mL PBS+GCRV病毒液)。GCRV是由野外采集的草鱼出血病患鱼匀浆制成(其比例为2 mL PBS:1 g 病鱼组织, 毒株编号为GCRV-JX0901)。采集各组草鱼血清和肠道样本, 用来分析饲料中添加大型海藻对感染GCRV的草鱼血清补体蛋白表达和肠道微生物的影响。

1.2 草鱼饲喂和样品采集

草鱼在广东联鲲集团有限公司研究院循环水系统进行饲养, 水温维持在28—30℃。草鱼每天投喂两次(9:00和16:00)。注射GCRV前, 立即采集草鱼的血清和肠道微生物样本(标记为G0), 之后分别采集GCRV注射后的1d、4d、7d、21d(标记为G1、G4、G7、和G21)的样本, 采集注射不含GCRV的PBS液的草鱼21d(标记为U21)的样本。采集方式:

每组随机挑选3尾草鱼, MS-222麻醉后抽血, 全血低温静置(4℃, 30min), 离心(3500 r/min, 15min)获得的血清立即置于-80℃保存。抽血后的草鱼在无菌条件下解剖, 取出肠道后液氮速冻, 运回实验室后保存于-80℃用于后续的肠道微生物DNA提取。

1.3 肠道微生物16S rRNA基因测序和补体蛋白测定

本实验获得的草鱼肠道样本, 使用PowerFecal™ DNA提取试剂盒(MoBio, CA, USA)提取肠道微生物DNA。使用细菌16S rRNA基因通用引物338F (5'-ACTCCTACGGGAGGCAGCA-3')和806R (5'-GGACTACHVGGGTWTCTAAT-3')扩增V3—V4区。扩增条件: 在94℃预变性1.5min; 25个循环的常规PCR扩增(94℃变性45s, 58℃退火30s, 72℃延伸60s); 最后72℃延伸10min, 最终4℃保存。利用1.2%的琼脂糖电泳对PCR产物进行切胶纯化, 使

表1 马尾藻、紫杉状海门冬和龙须菜的成分及其相对比例(%湿重)

检测项目 Item	马尾藻 <i>Sargassum hemiphyllum</i>	紫杉状海门冬 <i>Asparagopsis taxiformis</i>	龙须菜 <i>Gracilaria lemaneiformis</i>
粗蛋白Crude protein	7.75	13.98	17.22
粗脂肪Crude lipid	0.08	0.38	0.51
粗灰分 Crude ash	42.8	44.0	36.5
总磷Total phosphorus	0.11	0.09	0.18
淀粉Starch	0	1.1	0.4
粗纤维Crude fiber	4.4	5.8	5.9
氨基酸组成Amino acid composition			
天门冬氨酸Asp	0.78	1.60	1.59
苏氨酸Thr	0.35	0.83	0.73
丝氨酸Ser	0.30	0.70	0.65
谷氨酸Glu	0.85	1.48	1.61
甘氨酸Gly	0.38	0.76	0.82
丙氨酸Ala	0.45	1.03	0.89
缬氨酸Val	0.40	0.91	0.82
蛋氨酸Met	0.18	0.27	0.31
异亮氨酸Ile	0.32	0.73	0.68
亮氨酸Leu	0.54	1.12	1.04
酪氨酸Tyr	0.26	0.49	0.64
苯丙氨酸Phe	0.34	0.78	0.70
组氨酸His	0.10	0.07	0.17
赖氨酸Lys	0.35	0.66	0.70
精氨酸Arg	0.40	1.01	0.87
脯氨酸Pro	0.32	0.70	0.60
氨基酸总和 Sum of amino acids	6.32	13.14	12.82

用NEXTFLEX Rapid DNA-Seq Kit进行建库, 然后在Illumina HiSeq 3000测序平台进行测序(上海美吉生物医药科技有限公司)。通过Fastp对原始测序序列进行质控, 使用UCHIME算法和Uparse软件^[23], 识别并去除序列中的嵌合体并且将大于97的相似度阈值的序列聚类为同一个OTU (Operational taxo-

表 2 基础饲料配方及化学组成(%干物质)

Tab. 2 Formulation and chemical composition of the basal diet (% dry matter)

饲料原料Ingredient	分组Group			
	C	S	A	G
美国猪肉粉American pork powder	5.00	5.00	5.00	5.00
豆粕Soybean meal ¹	30.00	30.00	30.00	30.00
加籽菜粕Rapeseed meal ²	25.50	25.50	25.50	25.50
全脂米糠Rice bran ³	9.30	9.30	9.30	9.30
马尾藻 <i>S. hemiphyllum</i> ultrafine powder		5.00		
紫衫状海门冬 <i>A. taxiformis</i> ultrafine powder			5.00	
龙须菜 <i>G. lemaneiformis</i> ultrafine powder				5.00
高筋面粉Flour ⁴	24.00	19.00	19.00	19.00
豆油Soybean oil	3.00	3.00	3.00	3.00
草食性鱼预混料Premix (1%) ⁵	1.00	1.00	1.00	1.00
磷酸二氢钙Ca(H ₂ PO ₄) ₂	2.00	2.00	2.00	2.00
氯化胆碱Choline chloride (50%)	0.20	0.20	0.20	0.20
合计Total (%)	100.00	100.00	100.00	100.00
营养水平(测定值)Chemical composition (determined value)				
粗蛋白Crude protein	30.48	30.93	31.48	30.40
粗脂肪Crude lipid	6.09	6.00	6.01	6.01
钙Calcium	0.90	0.94	1.05	1.03
磷Phosphorus	1.32	1.31	1.31	1.30
赖氨酸Lysine	1.71	1.72	1.75	1.70
蛋氨酸Methionine	0.48	0.49	0.49	0.48

注: ¹豆粕46%粗蛋白; ²加籽菜粕36%粗蛋白; ³全脂米糠14%粗蛋白; ⁴高筋面粉14%粗蛋白; ⁵草食性鱼预混料(mg/kg): 维生素 A, 350; 维生素 D₃, 80; 维生素 E, 2500; 维生素 K₃, 320; 维生素 B₁, 400; 维生素 B₂, 430; 维生素 B₆, 420; 维生素 B₁₂, 3; 维生素 C, 3500; D-泛酸钙, 1250; 烟酰胺, 2500; 叶酸, 250; D-生物素, 6; 肌醇, 3000; 硫酸镁, 600; 硫酸锌, 1800; 硫酸锰, 1100; 硫酸铜, 1050; 硫酸亚铁, 11000; 硫酸钴, 120; 碘化钾, 90; 亚硒酸钠, 25; 主要原料由广东联鲲集团有限公司提供, 产地和加工方式不明

Note: ¹Soybean meal 46% crude protein; ²Rapeseed meal 36% crude protein; ³Rice bran 14% crude protein; ⁴Flour 14% crude protein; ⁵Premix (mg/kg): Vitamin A, 350; Vitamin D₃, 80; Vitamin E, 2500; Vitamin K₃, 320; Vitamin B₁, 400; Vitamin B₂, 430; Vitamin B₆, 420; Vitamin B₁₂, 3; Vitamin C, 3500; D-calcium pantothenate, 1250; Nicotinamide, 2500; Folic acid, 250; D-biotin, 6; Inositol, 3000; MgSO₄·H₂O, 600; ZnSO₄·7H₂O, 1800; MnSO₄·H₂O, 1100; CuSO₄·5H₂O, 1050; FeSO₄·H₂O, 11000; CoSO₄·7H₂O, 120; KI, 90; Na₂SeO₃, 25; The main raw materials are provided by Guangdong Nutriera Group Co. Ltd, the origin and processing method are unknown

nomie units)。依据Silva 16S rRNA数据库^[24], 通过RDP classifier对OTU进行物种注释。

此外, 将收集到的草鱼血清样本, 采用ELISA方式(试剂盒购自长沙奥基生物科技有限公司)分别测定C5、C5a和C5aR蛋白表达量。

1.4 统计分析

将原始丰度表进行抽平, 抽平后的各样本OTU数为23055条, 使用抽平后的OTU相对丰度表, 计算香农指数和辛普森指数, 各阶段Alpha多样性指数在SPSS 27.0 软件进行单因素方差分析(One-way ANOVA), 显著水平为 $P<0.05$, 之后使用Duncan's法进行多重比较。基于Bray-Curtis距离算法分析Beta多样性, 各组草鱼肠道微生物进行非度量多维尺度分析(NMDS), 使用Anosim和Adonis检验同组间感染和未感染的肠道微生物的差异。通过维恩图统计各组样品的共有和特有OTU。SPSS27.0软件分析比较各组样本获得显著性差异的物种。通过MENA (<http://ie-g4.rccc.ou.edu/MENA/>)在线平台对感染后草鱼肠道微生物群落进行共现网络分析^[25], 采用Gephi 0.10.1将结果进行可视化。使用Graph-Pad Prism 8软件对草鱼血清中C5、C5a和C5aR补体蛋白的浓度进行单因素方差分析(ANOVA), 显著水平为 $P<0.05$, 之后使用Tukey法进行多重比较。肠道微生物相对丰度变化与补体蛋白表达变化的相关性热图在派森诺基因云平台(<https://www.genesccloud.cn/home>)计算。使用欧易云平台(<https://www.cloud.oebiotech.com>)对PICRUSt 2 (版本5.0.4)样本微生物代谢通路的功能进行预测, 通过STAMP软件进行 Welch's *t*检验, 获得丰度均值显著差异的功能聚类。

2 结果

2.1 大型海藻对健康草鱼肠道微生物的影响

Alpha多样性分析显示, 相较于对照组, 大型海藻组草鱼肠道微生物差异显著(图 1c)。马尾藻和龙须菜显著提高了草鱼肠道菌群中厚壁菌门的相对丰度($P<0.05$; 图 1d)。韦恩图结果可知, 投喂海藻提高了草鱼肠道特有微生物的数量(图 1f)。热图展示了属水平相对丰度前二十的肠道微生物菌群在对照组和海藻组的富集程度(图 1e)。

2.2 大型海藻对感染GCRV后各组草鱼肠道微生物的影响

Shannon和Simpson指数表征菌群多样性, 以Pielou's 指数表征菌群均匀度, 以ACE表征菌群的丰富度。结果显示: 感染后0—7d, 各组的Alpha多样性指数均没有显著差异($P>0.05$), 但饲料中添加

大型海藻投喂的草鱼感染GCRV后0和1d肠道菌群Alpha多样性指数高于对照组, 随感染后时间的增加, 各组肠道菌群多样性总体上呈现由高降低再升高的趋势($P>0.05$; 表3)。对感染GCRV前第0天(G0)与感染后4—7d(G4&G7)的各组草鱼肠道微生物进行基于Bray-Curits距离的差异分析。结果显示, 对照组草鱼感染GCRV后4—7d肠道微生物菌群结构发生了显著改变(Anosim, Adonis $P<0.05$), 而海藻组未发生显著改变($P>0.05$; 表4)。进一步分析感染GCRV后4d和7d肠道微生物的差异物种, 在门水平上优势菌门为变形菌门(Proteobacteria)、梭杆菌门(Fusobacteriota)、拟杆菌门(Bacteroidota)、放线菌门(Actinobacteriota)、厚壁菌门(Firmicutes)、脱硫杆菌门(Desulfobacterota)和疣微菌门(Verrucomicrobiota; 图2a)。在属水平, 感染GCRV后4—

7d各组草鱼肠道菌群相对丰度前十的属依次为鲸杆菌属(*Cetobacterium*)、弧菌属(*Vibrio*)、气单胞菌属(*Aeromonas*)、脱硫杆菌属科未分类属(*g_unclassified_f_Desulfovibrionaceae*)、分支杆菌属(*Mycobacterium*)、拟杆菌属(*Bacteroides*)、*g_unclassified_f_Mycoplasmataceae*、迪茨氏菌属(*Dietzia*)、屠场杆菌属(*Macellibacteroides*; 图2b)。感染后4d, 马尾藻组和龙须菜组草鱼肠道中弧菌属相对丰度显著降低(Duncan's; $P<0.05$; 图2c), 感染后7d, 马尾藻组的梭杆菌门鲸杆菌属的相对丰度显著降低($P<0.05$; 图2d和2e), 而促进了拟杆菌属的相对丰度增加($P<0.05$; 图2f)。

2.3 感染GCRV后草鱼肠道微生物共现网络分析

通过基于随机矩阵理论(RMT)的方法构建名为分子生态网络(MENA)的生态关联网络, 用于了

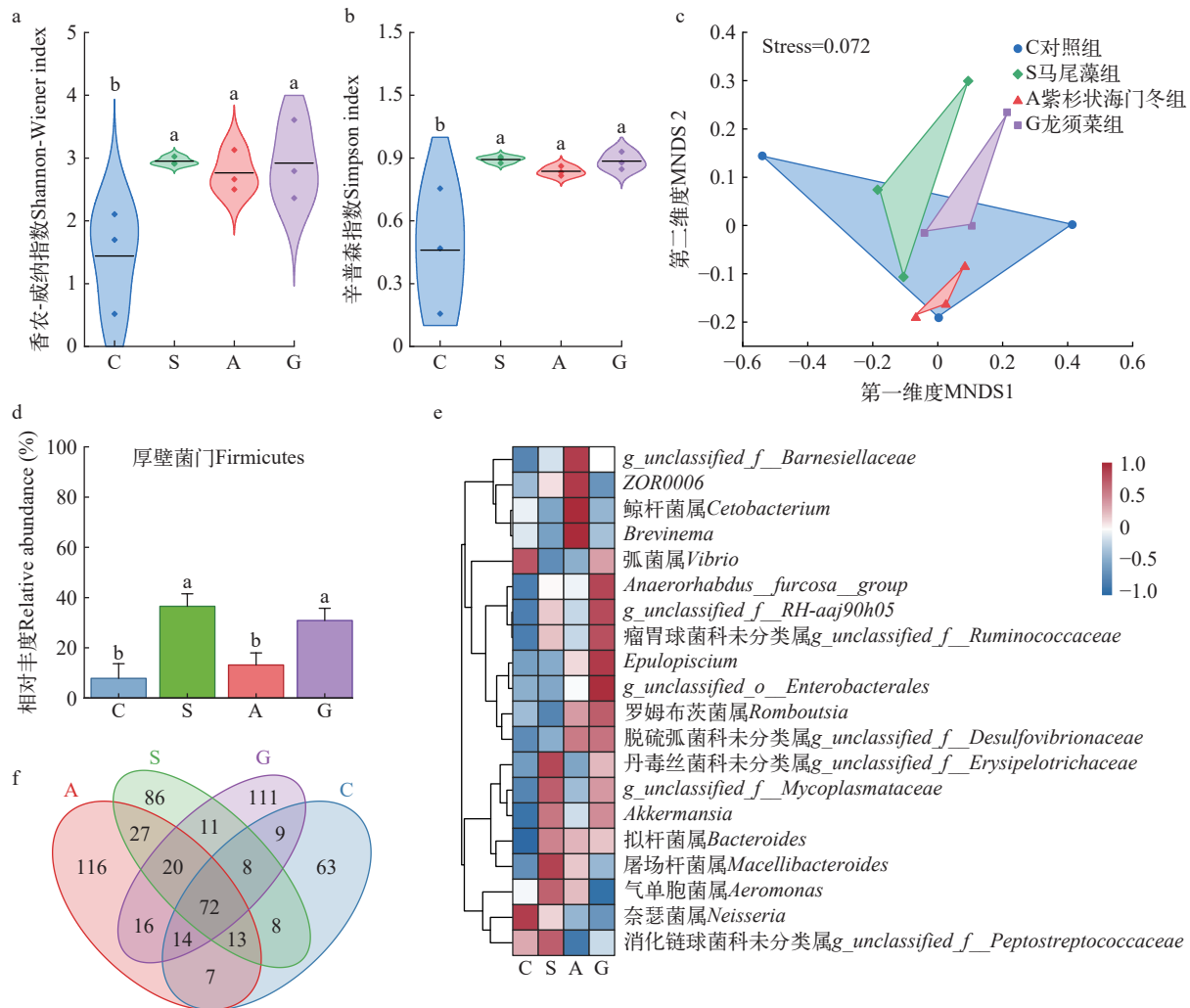


图1 感染对照组21d (U21)肠道微生物多样性和菌群差异分析

Fig. 1 Analysis of gut microbiota diversity and flora difference on day 21 (U21) of infection control group

a. 香农指数; b. 辛普森指数; c. NMDS分析; d. 门水平差异物种; e. 属水平热图; f. OTU水平韦恩图

a. Shannon index; b. Simpson index; c. NMDS analysis; d. Phyla level difference species; e. Genus-level heat map; f. Venn diagram of OTU level

表 3 GCRV感染前后肠道微生物Alpha多样性差异

Tab. 3 Differences in gut microbiota Alpha diversity before and after GCRV infection (mean±SD, n=3)

天数Day	多样性Alpha	对照组C	马尾藻组S	紫杉状海门冬组A	龙须菜组G
G0	Shannon	2.28±0.48	2.35±1.08	2.32±0.23	3.05±0.73
	Simpson	0.82±0.06	0.74±0.15	0.79±0.07	0.90±0.06
	Pielou's	0.54±0.07	0.49±0.15	0.50±0.05	0.65±0.09
	ACE	67.69±21.91	129.55±93.43	110.4±44.49	120.11±67.64
G1	Shannon	1.70±1.21	1.86±1.20	2.28±0.99	2.29±0.27
	Simpson	0.59±0.38	0.68±0.39	0.73±0.22	0.78±0.08
	Pielou's	0.40±0.24	0.43±0.24	0.49±0.15	0.52±0.04
	ACE	63.1±38.49	75.56±38.31	127.15±63.44	86.1±15.53
G4	Shannon	2.51±0.48	1.71±0.58	2.47±0.48	2.21±0.94
	Simpson	0.82±0.06	0.64±0.18	0.82±0.10	0.73±0.23
	Pielou's	0.55±0.07	0.41±0.13	0.55±0.09	0.47±0.17
	ACE	98.88±30.67	71.72±21.86	97.97±28.26	103.35±42.82
G7	Shannon	2.39±0.60	2.01±0.27	2.08±0.85	2.43±0.48
	Simpson	0.79±0.08	0.73±0.05	0.69±0.28	0.81±0.07
	Pielou's	0.53±0.08	0.46±0.05	0.45±0.16	0.54±0.08
	ACE	97.34±50.44	80.81±11.90	96.44±30.44	98.73±39.05

注: 表中数据为3个重复的平均值, 同行数据不同上标字母表示差异显著($P<0.05$)

Note: The data is the average of three replicates, and different superscript letters in the same row indicate significant differences ($P<0.05$)

表 4 GCRV感染前与感染4d和7d肠道微生物的Bray-Curits距离差异

Tab. 4 Differences in Bray-Curits distance of gut microbes before GCRV infection and on days 4 and 7 of infection

分组 Group	对比 Comparison	Anosim		Adonis	
		R	P	R	P
对照组C	G0 vs. G4&G7	0.376	0.03*	1.93	0.045*
马尾藻组S	G0 vs. G4&G7	0.209	0.155	1.34	0.213
紫杉状海门冬组A	G0 vs. G4&G7	0.209	0.126	1.59	0.157
龙须菜组G	G0 vs. G4&G7	0.117	0.186	1.34	0.131

注: “*” $P<0.05$

Note: “*” represents $P<0.05$

解群落内不同物种之间的相互作用及其对环境变化的反应。结果显示感染GCRV后1—7d促使各组草鱼肠道菌群之间主要以负相关为主。厚壁菌门、变形菌门和拟杆菌门成为各组共现网络的主要菌门(图 3)。以网络节点数、边的数量、平均聚合度、平均聚类系数和网络密度表征共现网络的复杂性(表 5), 饲料中添加大型海藻投喂草鱼在感染GCRV后, 肠道微生物共现网络复杂度高于对照组。

2.4 感染GCRV后草鱼血清中补体蛋白结果

草鱼注射感染GCRV后, 血清中C5蛋白含量呈现出先升高后降低的趋势, 而C5a和C5aR含量主要在感染1d呈现上调表达, 之后随时间推移缓慢降低至正常水平(图 4)。感染后21d, 马尾藻显著提高了草鱼血清中C5蛋白浓度(Tukey多重比较, $P<0.05$)。

感染后1d, 马尾藻显著上调了草鱼血清中C5a的含量, 而龙须菜组则C5a蛋白含量降低($P<0.05$)。这表明马尾藻可能促进了草鱼感染GCRV后血清中C5a的表达, 而龙须菜则表现为抑制作用。未观察到感染后各大型海藻对草鱼血清中C5aR的改变, 结果显示大型海藻未对C5aR的表达造成影响($P>0.05$)。

2.5 感染后草鱼肠道微生物功能预测分析

使用PICRUST2软件对各组草鱼肠道微生物在感染后1—7d的菌群功能进行预测, 并进行KEGG Pathway的功能注释。在Level 2预测后, 将代谢、遗传信息处理、细胞过程、环境信息处理这4类功能进行STAMP差异分析, Welch's *t*检验, 获得丰度均值显著差异的功能聚类(图 5)。结果显示马尾藻和龙须菜组肠道菌群在氟苯甲酸盐的降解都显著增强, 马尾藻和紫杉状海门冬组在细胞色素P450对外源性药物的代谢途径显著增强。马尾藻组肠道菌群对乙醛酸盐和二羟酸盐代谢增强, 紫杉状海门冬组肠道菌群对萘降解途径显著增强, 龙须菜组草鱼对缬氨酸、亮氨酸和异亮氨酸的降解显著增强。总体上3种大型海藻肠道微生物群落对酸碱盐和卤素化合物的降解, 氨基酸代谢的能力显著增强。

2.6 草鱼补体蛋白表达与肠道微生物相关性分析

将对照组和海藻组补体表达具有显著差异阶段(G1和G21)的肠道菌群与对应的补体表达量进行斯皮尔曼相关性分析发现, 草鱼补体蛋白表达水平

与肠道菌群相对丰度之间存在显著相关性(图 6)。草鱼血清中C5、C5a和C5aR表达量与微小杆菌属(*Exiguobacterium*)、不动杆菌属(*Acinetobacter*)、*Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*、红球菌属(*Rhodococcus*)、短波单胞菌属(*Brevundimonas*)呈显著正相关($P < 0.05$)。除C5与螺旋菌属(*Brevinema*)无显著相关性外($P > 0.05$)，C5和C5a表达量与ZOR0006、[*Anaerorhabdus*]*_furcosa_group*、螺旋菌属、*g_uncultured_f_Barnesiellaceae*、丹毒杆菌属(*Erysipelatoclostridium*)、*Akkermansia*、*Parabacteroides*呈显著负相关($P < 0.05$)。

3 讨论

3.1 大型海藻显著增加草鱼肠道菌群的多样性和特有菌群数量

有研究报道,大型海藻含有多种天然碳水化合物,作为益生元能够刺激肠道菌群增殖、提高肠道微生物多样性^[26]。在本研究中,大型海藻显著增加了健康草鱼肠道微生物的多样性,改变了肠道菌群结构。这与张露等^[27]报道的红藻门紫菜显著增加大黄鱼肠道微生物多样性和Rico等^[28]报道的褐藻门江篱提高金头鲷肠道微生物多样性的结果一致。此外,马尾藻和龙须菜显著提高了健康草鱼肠

道中厚壁菌门的相对丰度,与Chen等^[29]研究褐藻发酵菌群促进厚壁菌群增加的结果一致。热图和韦恩图表明大型海藻增加了以膳食纤维发酵和碳水化合物消化为主的特有菌群种类的数量^[30-34]。大型海藻营养成分检测表显示,3种大型海藻相较于高筋面粉具有较高的粗纤维含量,且含有丰富的矿物质。大型海藻膳食纤维具有孔状结构能够成为发酵菌群和以消化碳水化合物菌群为主的肠道微生物的栖息地^[35,36],同时,丰富的矿物质含量也提高了草鱼肠道微生物的多样性和特有菌群种类的数量,而促进了肠道的消化吸收和动物对抗疾病的能力^[37]。

3.2 大型海藻可缓解感染草鱼肠道菌群紊乱促进有益菌群增加

本研究发现感染GCRV的各组草鱼间肠道菌群Alpha多样性无显著差异,但饲料中添加大型海藻投喂提高了感染草鱼0—1d肠道菌群的多样性。随着感染时间的推移,各组肠道菌群多样性总体上呈现由高降低再升高的趋势。与Xiao等^[9]研究GCRV感染草鱼发病时间的趋势一致。这表明GCRV感染草鱼随病程发展,严重降低了草鱼肠道微生物的多样性,而添加大型海藻有利于提高草鱼肠道菌群多样性,减缓了感染草鱼肠道菌群多样性的降低。

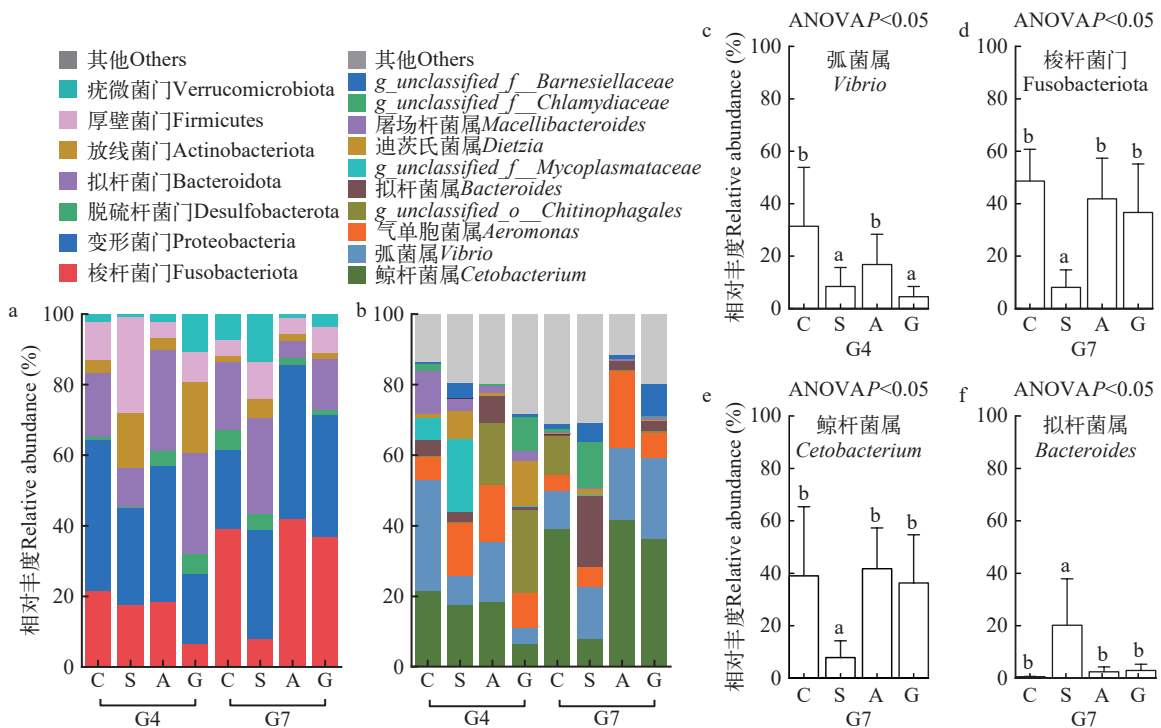


图2 感染GCRV4d和7d肠道微生物菌群相对丰度及差异

Fig. 2 Relative abundance and difference of gut microbiota on days 4 and 7 after GCRV infection

a. 门水平; b. 属水平; c—d. 感染4d; e—f. 感染7d

a. phylum level; b. genus level; c—d. day 4 of infection; e—f. day 7 of infection

已有研究报道, 草鱼感染GCRV后4—7d是肠道菌群发生紊乱的主要时间段^[9], 因此本研究重点关注感染后4—7d的菌群结构变化。基于Bray-Curits距

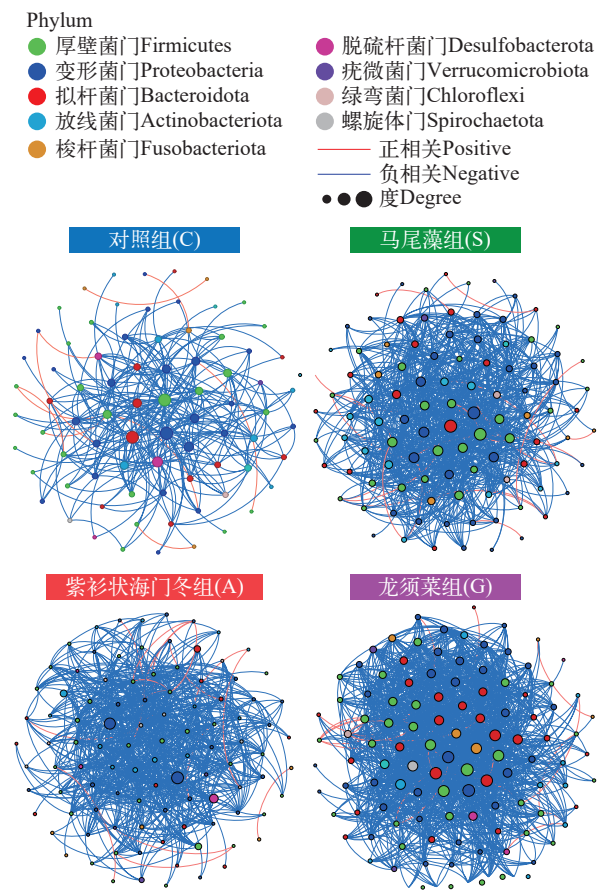


图3 感染GCRV后各组肠道微生物共现网络比较

Fig. 3 Comparison of the co-occurrence network of gut microbiota in each group after GCRV infection

节点的大小与边的连接数量成正比(即为: 度), 节点的不同颜色表示不同菌门; 红色、蓝色连线分别表示微生物之间呈正相关和负相关

The size of nodes is proportional to the number of connected edges (i.e., degree), and different colors of nodes indicate different phylum. Red and blue lines indicate positive and negative correlations between microorganisms, respectively

表5 GCRV感染后草鱼肠道微生物共现网络的拓扑学参数

Tab. 5 Topological parameters of gut microbiota co-occurrence network in grass carp after GCRV infection

组别 Group	对照组 C	马尾藻组 S	紫衫状海 门冬组A	龙须菜组 G
节点数量Node	78	103	101	111
边的数量Link	259	885	836	1357
平均聚合度Avg K	6.641	17.184	16.554	24.45
平均聚类系数Avg CC	0.058	0.236	0.265	0.219
网络密度Density	0.086	0.168	0.166	0.222
正相关Positive correlation	3.86	3.24	0.98	2.10
负相关Negative correlation	96.14	96.76	99.02	97.90

离进行两组间差异检验显示, 对照组草鱼注射感染GCRV前与注射感染后4—7d肠道菌群组成差异显著。这与朱文根等^[38]研究草鱼感染GCRV后肠道菌群发生显著变化的结果类似。感染GCRV后草鱼消化系统功能受到损害^[39], 导致草鱼食欲减退甚至停止摄食而诱发肠道微生物定植的环境恶化, 肠道菌群发生显著改变^[10, 40]。相反, 饲料中添加大型海藻投喂的草鱼在感染GCRV后, 感染前与感染后4—7d相比肠道微生物群落结构未发生显著改变, 表明大型海藻降低了草鱼感染GCRV后肠道菌群的紊乱。与Yao等^[41]研究海藻提取物海藻酸盐可以修复腹泻小鼠肠道菌群紊乱的结果类似, 可能是海藻含有较多的膳食纤维和多种藻类多糖及矿物元素改变了肠道菌群结构, 增强了菌群对病原微生物的抵御能力^[42, 43]。进一步探究感染后4—7d各组草鱼肠道菌群的变化。草鱼感染GCRV后4d, 马尾藻和龙须菜显著降低草鱼肠道中弧菌属的相对丰度。宿主感染病原微生物后可引起肠道菌群紊乱^[44], 而大型海藻在动物体内具有抑制有害菌并调节肠道菌群组成的功能^[45, 46]。弧菌属是水产动物中高致病性微生物^[47], 与马尾藻投喂对虾^[48]、龙须菜投喂黄斑蓝子鱼^[49]抑制肠道菌群中弧菌属的结果一致, 可能是马尾藻和龙须菜的具有较强抗菌活性, 在感染期能够抑制部分有害菌的生长^[50]。在感染GCRV后7d, 马尾藻组草鱼肠道菌群中梭杆菌门鲸杆菌属的相对丰度减少, 拟杆菌属的相对丰度增加。与Xie等^[51]用马尾藻提取物饲喂小鼠后肠道菌群拟杆菌属显著增加的结果一致。拟杆菌属是草鱼肠道菌群中有益于消化膳食纤维和维持菌群稳定的优势菌群^[31], 饲料中添加含有较多膳食纤维的马尾藻投喂草鱼促进了拟杆菌属的增加, 也表明大型海藻中膳食纤维有利于维持感染GCRV草鱼肠道有益菌群的稳定。

3.3 大型海藻增强感染GCRV草鱼的肠道菌群的稳定性

肠道中的菌群之间存在着复杂的相互作用和相互影响的关系, 构建形成一个庞大的生态关联网络^[52], 通过共现网络分析能够反映出这种菌群关联网络在应对疾病干扰时所产生的变化^[53, 54]。在本研究中, 感染GCRV后各组草鱼肠道微生物共现网络主要以负相关为主。与Liu等^[55]报道淡水腹足动物对抗有毒藻类侵害时, 肠道微生物网络以负相关为主的结果一致。菌群之间竞争加剧是微生物之间应对不良环境菌群关系转变的一种方式^[56], 有利于菌群网络的稳定。此外, 共现网络显示饲料中添加大型海藻投喂的草鱼肠道微生物网络复杂度较

高, 饲料中添加大型海藻投喂草鱼丰富了肠道菌群之间的影响关系, 菌群节点数和连接数量明显增加, 菌群之间形成了密集的关联网络。研究表明, 高复杂度的肠道菌群网络对抗扰动环境更加稳定, 有助于菌群抵御不良环境的扰动^[57]。从网络组成菌群种类发现, 厚壁菌门是菌群网络组成的优势菌门, 表明大型海藻有利于厚壁菌门菌群的增殖从而建立复杂的菌群关联网络, 可能强化了草鱼肠道屏障功能而减轻了肠道炎症对菌群网络的影响^[58], 促进了肠道菌群的稳定以对抗GCRV的感染。

3.4 大型海藻影响草鱼感染GCRV后的补体表达水平

草鱼感染GCRV后, C5、C5a和C5aR由肝脏大量合经血液运输至全身引发机体炎症反应^[59], 大型海藻作为膳食补充可提高鱼类免疫力^[18], 增强机体对疾病的抵御。在本研究中, 各组感染草鱼血清中补体C5浓度呈现出先升高后降低的趋势, 与吕丽刚^[60]报道的感染GCRV的草鱼肝脏中的补体C5基因表达的结果一致。而各组草鱼血清中的C5a含量在感染后1d达到最高峰, C5aR浓度在感染后无显著改变。与苏杭^[61]报道的感染GCRV的草鱼C5a和C5aR在肝脏中表达高峰期在4—5d和3d存在差异。这与

感染的草鱼规格有关, 在草鱼感染GCRV的1年鱼种中, 较大个体的草鱼免疫系统发育趋于完善, 能够快速应对病毒感染而做出免疫反应^[62]。补体C5裂解为C5a成分, C5a作为引发机体炎症反应的强过敏毒素^[63], 其表达上调意味着机体炎症反应激烈^[64], C5a/C5aR作为促炎的主要通路参与调节机体炎症反应^[65]。马尾藻感染后1d和21d显著上调了草鱼血清中C5a和C5的浓度, 而龙须菜组草鱼则在感染1d后血清中C5a的含量降低。这表明马尾藻可能促进炎症发生, 龙须菜可能有效缓解了草鱼感染GCRV后炎症的产生。有研究表明, 马尾藻经草鱼肠道微生物消化产生的乙酸等短链脂肪酸等物质与促进了炎症的发生有关^[66]。本研究对感染后草鱼肠道菌群进行功能预测发现, 马尾藻显著增强了肠道菌群对乙酸盐物质的代谢, 可能是导致C5a补体蛋白表达上调的原因。龙须菜含有丰富的龙须菜多糖和多种氨基酸, 并含有大量的矿物元素^[67], 经过消化分解后会释放出较多卤素化合物^[68]。代谢通路显示龙须菜组草鱼肠道菌群对卤素化合物和氨基酸的代谢显著增强, 这些活性物质的代谢有助于调节动物免疫表达^[69], 从而改变GCRV侵染引起的炎症反应, 可能是引起C5a的含量降低的原因

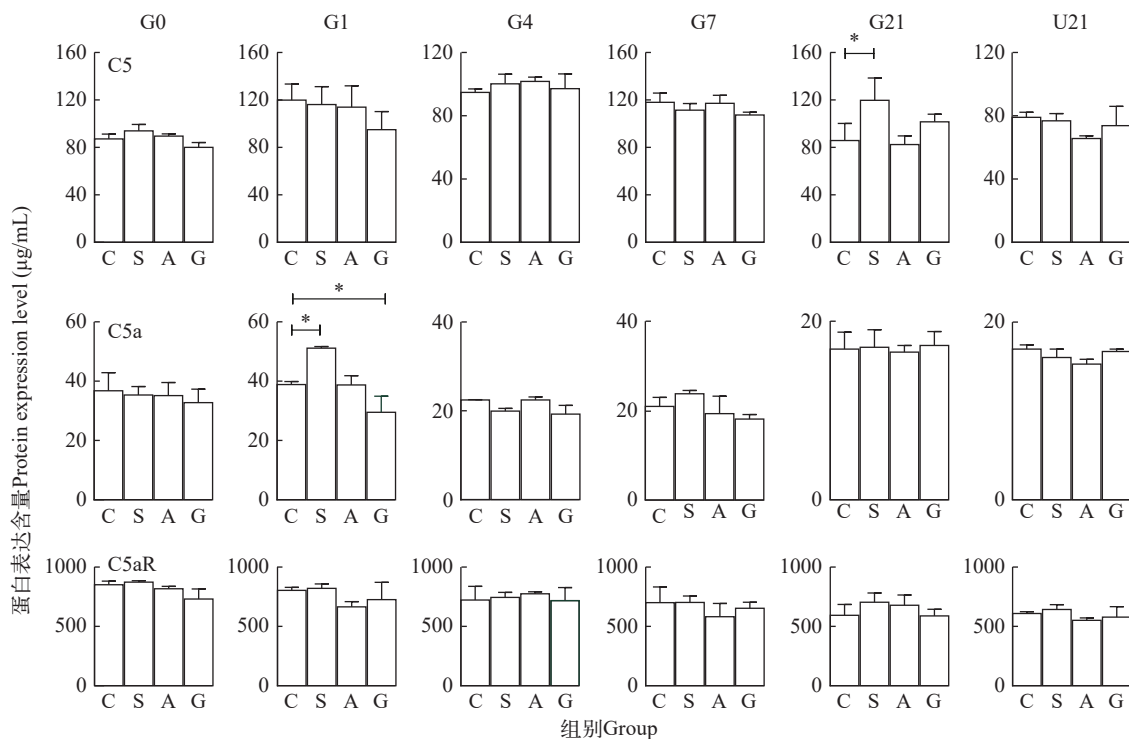


图4 不同组别草鱼补体蛋白含量比较

Fig. 4 Complement protein concentrations in different groups of grass carp

G0、G1、G4、G7和G21分别表示注射GCRV 0、1d、4d、7d、21d; U21表示注射PBS液后21d; * $P < 0.05$

0, 1d, 4d, 7d, and 21d after injection of GCRV containing PBS solution are denoted as G0, G1, G4, G7, and G21, respectively. Day 21 after injection of PBS solution without GCRV is indicated as U21; * $P < 0.05$

之一。

3.5 大型海藻调控肠道微生物影响补体蛋白的表达

微生物定植在肠道中通过相互影响形成了关系复杂的微生物菌群,与宿主免疫表达存在相互调控的作用^[70-72]。本研究显示,感染GCRV后的补体C5、C5a和C5aR的表达与微小杆菌属、不动杆、*Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*、红球菌属、短波单胞菌呈正相关。其中,微

小杆菌属和不动杆是导致鱼类致病的有害菌群^[73],*Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*易导致肠炎^[74],红球菌属在鱼肠道中主要消化难降解物质的物质^[75],短波单胞菌为鱼类条件致病菌^[76]。这表明补体C5、C5a和C5aR的上调表达多与大型海藻调节肠道中有害菌的增殖有关。与Ikeda-Ohtsubo等^[77]发现海藻提取的岩藻聚糖投喂斑马鱼可以降低肠杆菌属的相对丰度而降低IL-

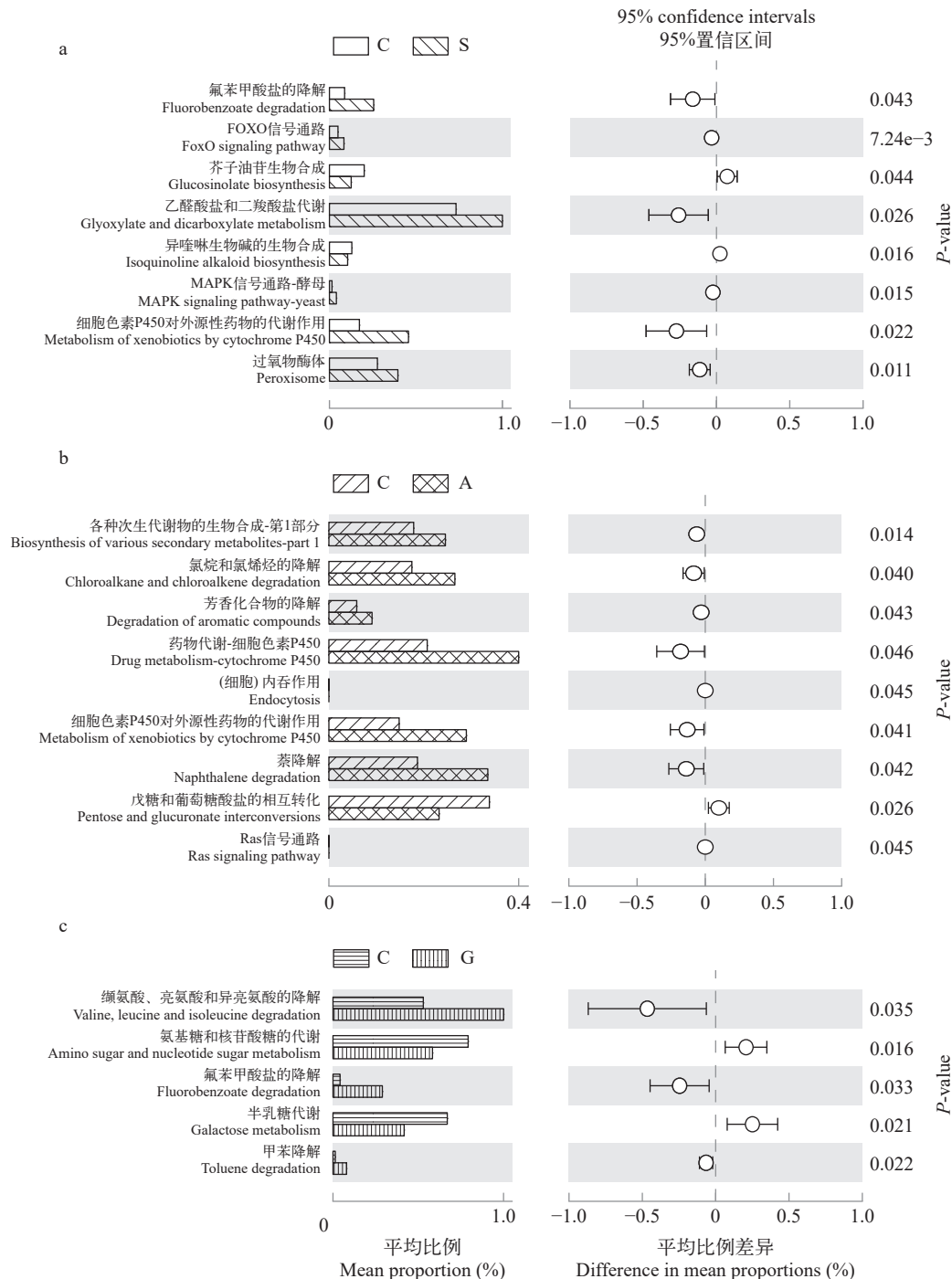


图 5 感染GCRV后草鱼肠道微生物功能预测

Fig. 5 Prediction of gut microbes function in grass carp infected with GCRV

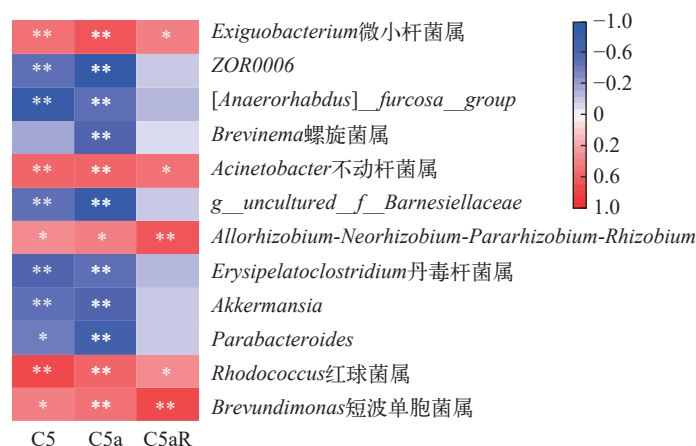


图6 C5、C5a、C5aR与肠道微生物相对丰度的相关性分析

Fig. 6 Correlation analysis of C5, C5a, and C5aR with the relative abundance of gut microbes (* $P < 0.05$, ** $P < 0.01$)

1 β 的表达结果相类似。而ZOR0006、[*Anaerorhabdus*]*_furcosa_group*、螺旋菌属^[32]、*g_ uncultured_f_ Barnesiellaceae*、丹毒杆菌属^[33]、*Akkermansia*和*Parabacteroides*^[34]是草鱼肠道菌群碳水化合物分解和膳食纤维发酵消化的优势种群。C5和C5a的表达与消化分解碳水化合物和膳食纤维菌群的相对丰度呈现负相关,表明草鱼产生严重炎症状态的情况下不利于有益微生物消化分解碳水化合物和膳食纤维菌群的增殖;暗示着草鱼肠道中这些消化菌群的稳定增殖可以抑制感染GCRV草鱼炎症的产生,有助于草鱼抵抗疾病感染。

4 结论

饲料中添加大型海藻投喂草鱼显著提高了肠道菌群的多样性和特有微生物数量,有效减缓了GCRV感染1d后的草鱼肠道菌群多样性的降低,缩小了GCRV感染4—7d草鱼肠道菌群的组间差异,有助于降低GCRV感染引起的肠道菌群紊乱。其中,马尾藻和龙须菜减少了感染草鱼肠道中有害菌群弧菌属的相对丰度,马尾藻促进了益生菌拟杆菌属的相对丰度。此外,大型海藻提高了感染草鱼肠道菌群网络的复杂性,增强了肠道菌群的稳定性。蛋白表达结果显示,马尾藻使草鱼血清中促炎因子补体成分C5a含量增加,而龙须菜则降低血清中C5a的浓度,趋于抑制炎症的产生。在草鱼感染GCRV的模型中,C5、C5a和C5aR在血清中的含量与肠道中致病菌群相对丰度的增加呈正相关,与碳水化合物和膳食纤维消化菌群呈负相关。综上,本研究初步阐述了饲料中添加大型海藻可以缓解GCRV感染对草鱼肠道菌群的不利影响,并展示了大型海藻对感染草鱼补体表达的调控,为探究大型海藻对感染草鱼肠道微生物的影响提供了基础数

据。目前缺乏有关大型海藻对草鱼起关键作用的活性物质的研究,还需进一步探索大型海藻对缓解草鱼肠道炎症的机制。

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参考文献:

- [1] Wang D, Wu F X, Song D D, *et al.* China Fisheries Statistical Yearbook [M]. Beijing: China Agricultural Press, 2023: 25. [王丹, 吴反修, 宋丹丹, 等. 中国渔业统计年鉴 [M]. 北京: 中国农业出版社, 2023: 25.]
- [2] Zeng W, Bergmann S M, Dong H, *et al.* Identification, virulence, and molecular characterization of a recombinant isolate of grass carp reovirus genotype I [J]. *Viruses*, 2021, **13**(5): 807.
- [3] Xiong L, He L, Luo L, *et al.* Global and complement gene-specific DNA methylation in grass carp after grass carp reovirus (GCRV) infection [J]. *International Journal of Molecular Sciences*, 2018, **19**(4): 1110.
- [4] Chen G, Xiong L, Wang Y, *et al.* Different responses in one-year-old and three-year-old grass carp reveal the mechanism of age restriction of GCRV infection [J]. *Fish & Shellfish Immunology*, 2019(86): 702-712.
- [5] Wu M, Li H, Chen X, *et al.* Studies on the clinical symptoms, virus distribution, and mRNA expression of several antiviral immunity-related genes in grass carp after infection with genotype II grass carp reovirus [J]. *Archives of Virology*, 2020, **165**(7): 1599-1609.
- [6] Yang G, Jian S Q, Cao H, *et al.* Changes in microbiota along the intestine of grass carp (*Ctenopharyngodon idella*): community, interspecific interactions, and functions [J]. *Aquaculture*, 2019(498): 151-161.
- [7] Medina-Félix D, Garibay-Valdez E, Vargas-Albore F, *et al.* Fish disease and intestinal microbiota: a close and indivisible relationship [J]. *Reviews in Aquaculture*, 2023, **15**(2): 820-839.
- [8] Zhang B Y, Yang H L, Cai G H, *et al.* The interactions

- between the host immunity and intestinal microorganisms in fish [J]. *Applied Microbiology and Biotechnology*, 2024, **108**(1): 30.
- [9] Xiao F, Liao L, Xu Q, *et al.* Host-microbiota interactions and responses to grass carp reovirus infection in *Ctenopharyngodon idellus* [J]. *Environmental Microbiology*, 2021, **23**(1): 431-447.
- [10] Xiong J B, Nie L, Chen J. Current understanding on the roles of gut microbiota in fish disease and immunity [J]. *Zoological Research*, 2019, **40**(2): 70-76.
- [11] Dunkelberger J R, Song W C. Complement and its role in innate and adaptive immune responses [J]. *Cell Research*, 2010, **20**(1): 34-50.
- [12] Rittirsch D, Flierl M A, Nadeau B A, *et al.* Functional roles for C5a receptors in sepsis [J]. *Nature Medicine*, 2008, **14**(5): 551-557.
- [13] Magnadóttir B. Innate immunity of fish (overview) [J]. *Fish & Shellfish Immunology*, 2006, **20**(2): 137-151.
- [14] Holland M C H, Lambiris J D. The complement system in teleosts [J]. *Fish & Shellfish Immunology*, 2002, **12**(5): 399-420.
- [15] Xu B, Su H, Chen C, *et al.* Pro-inflammatory regulatory mechanism of C5a/C5aR pathway in GCRV-infected grass carp (*Ctenopharyngodon idella*) [J]. *Aquaculture Reports*, 2023(32): 101736.
- [16] Xu B, Lv L, Xiao T, *et al.* Cloning of the full-length cDNA of the gene encoding complement C5 from grass carp (*Ctenopharyngodon idella*) and its expression in different tissues by following grass carp reovirus infection [J]. *Aquaculture International*, 2021(29): 2035-2048.
- [17] Rebours C, Marinho-Soriano E, Zertuche-González J A, *et al.* Seaweeds: an opportunity for wealth and sustainable livelihood for coastal communities [J]. *Journal of Applied Phycology*, 2014, **26**(5): 1939-1951.
- [18] Siddik M A B, Francis P, Rohani M F, *et al.* Seaweed and seaweed-based functional metabolites as potential modulators of growth, immune and antioxidant responses, and gut microbiota in fish [J]. *Antioxidants*, 2023, **12**(12): 2066.
- [19] Gupta S, Abu-Ghannam N. Bioactive potential and possible health effects of edible brown seaweeds [J]. *Trends in Food Science & Technology*, 2011, **22**(6): 315-326.
- [20] O'Sullivan L, Murphy B, McLoughlin P, *et al.* Prebiotics from marine macroalgae for human and animal health applications [J]. *Marine Drugs*, 2010, **8**(7): 2038-2064.
- [21] Priyanka K R, Rajaram R, Sivakumar S R. A critical review on pharmacological properties of marine macroalgae [J]. *Biomass Conversion and Biorefinery*, 2022: 1-25.
- [22] Wan A H L, Davies S J, Soler-Vila A, *et al.* Macroalgae as a sustainable aquafeed ingredient [J]. *Reviews in Aquaculture*, 2019, **11**(3): 458-492.
- [23] Edgar R C. UPARSE: highly accurate OTU sequences from microbial amplicon reads [J]. *Nature Methods*, 2013, **10**(10): 996-998.
- [24] Pruesse E, Quast C, Knittel K, *et al.* SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB [J]. *Nucleic Acids Research*, 2007, **35**(21): 7188-7196.
- [25] Deng Y, Jiang Y H, Yang Y, *et al.* Molecular ecological network analyses [J]. *BMC Bioinformatics*, 2012(113): 1-20.
- [26] Zhang H, Jiang F, Zhang J, *et al.* Modulatory effects of polysaccharides from plants, marine algae and edible mushrooms on gut microbiota and related health benefits: a review [J]. *International Journal of Biological Macromolecules*, 2022(204): 169-192.
- [27] Zhang L, Xiong Y M, Dong L L, *et al.* Effects of seaweed enzymatic hydrolysates on growth and intestinal microflora structure of *Larimichthys crocea* [J]. *Chinese Journal of Animal Nutrition*, 2024, **36**(3): 1819-1833. [张露, 熊宇明, 董黎黎, 等. 海藻酶解物对大黄鱼生长和肠道菌群结构的影响 [J]. 动物营养学报, 2024, **36**(3): 1819-1833.]
- [28] Rico R M, Tejedor-Junco M T, Tapia-Paniagua S T, *et al.* Influence of the dietary inclusion of *Gracilaria cornea* and *Ulva rigida* on the biodiversity of the intestinal microbiota of *Sparus aurata* juveniles [J]. *Aquaculture International*, 2016(24): 965-984.
- [29] Chen A, Liu Y, Zhang T, *et al.* Chain conformation, mucoadhesive properties of fucoidan in the gastrointestinal tract and its effects on the gut microbiota [J]. *Carbohydrate Polymers*, 2023(304): 120460.
- [30] Ai C, Jiang P, Liu Y, *et al.* The specific use of alginate from *Laminaria japonica* by *Bacteroides* species determined its modulation of the *Bacteroides* community [J]. *Food & Function*, 2019, **10**(7): 4304-4314.
- [31] Wang S T, Meng X Z, Dai Y F, *et al.* Characterization of the intestinal digesta and mucosal microbiome of the grass carp (*Ctenopharyngodon idella*) [J]. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, 2021(37): 100789.
- [32] Xu W, Lutz C G, Taylor C M, *et al.* Improvement of fish growth and metabolism by oligosaccharide prebiotic supplement [J]. *Aquaculture Nutrition*, 2022(2022): 5715649.
- [33] Beisner J, Gonzalez-Granda A, Basrai M, *et al.* Fructose-induced intestinal microbiota shift following two types of short-term high-fructose dietary phases [J]. *Nutrients*, 2020, **12**(11): 3444.
- [34] Liu Z, Hu Y, Tao X, *et al.* Metabolites of sea cucumber sulfated polysaccharides fermented by *Parabacteroides distasonis* and their effects on cross-feeding [J]. *Food Research International*, 2023(167): 112633.
- [35] Kong Q, Zhang R, You L, *et al.* In vitro fermentation characteristics of polysaccharide from *Sargassum fusiforme* and its modulation effects on gut microbiota [J].

- Food and Chemical Toxicology*, 2021(151): 112145.
- [36] Sun Y, Zhang C, Zhang P, *et al.* Digestion characteristics of polysaccharides from *Gracilaria lemaneiformis* and its interaction with the human gut microbiota [J]. *International Journal of Biological Macromolecules*, 2022(213): 305-316.
- [37] Rabesandratana T. 'Poop vault' of human feces could preserve gut's microbial biodiversity-and help treat disease [J]. *Science*, 2018: eaav9276.
- [38] Zhu W G, Li X H, Rao L Y, *et al.* Effects of reovirus infection on the intestinal microbiota diversity of grass carp (*Ctenopharyngodon idella*) [J]. *Acta Hydrobiologica Sinica*, 2019, **43**(1): 109-116. [朱文根, 李星浩, 饶刘瑜, 等. 感染草鱼呼肠孤病毒对肠道菌群多样性的影响 [J]. *水生生物学报*, 2019, **43**(1): 109-116.]
- [39] Parshukov A N, Kashinskaya E N, Simonov E P, *et al.* Variations of the intestinal gut microbiota of farmed rainbow trout, *Oncorhynchus mykiss* (Walbaum), depending on the infection status of the fish [J]. *Journal of Applied Microbiology*, 2019, **127**(2): 379-395.
- [40] Li X, Zhou L, Yu Y, *et al.* Composition of gut microbiota in the gibel carp (*Carassius auratus gibelio*) varies with host development [J]. *Microbial Ecology*, 2017, **74**(1): 239-249.
- [41] Yao L, Yang P, Lin Y, *et al.* The regulatory effect of alginate on ovalbumin-induced gut microbiota disorders [J]. *Journal of Functional Foods*, 2021(86): 104727.
- [42] Wang J, Zhang Z, Dai T, *et al.* The therapeutic effect and possible mechanisms of alginate oligosaccharide on metabolic syndrome by regulating gut microbiota [J]. *Food & Function*, 2024, **15**(19): 9632-9661.
- [43] Praveen M A, Karthika Parvathy K R, Balasubramanian P, *et al.* An overview of extraction and purification techniques of seaweed dietary fibers for immunomodulation on gut microbiota [J]. *Trends in Food Science & Technology*, 2019(92): 46-64.
- [44] Zeevi D, Korem T, Godneva A, *et al.* Structural variation in the gut microbiome associates with host health [J]. *Nature*, 2019, **568**(7750): 43-48.
- [45] Keating C, Bolton-Warberg M, Hinchcliffe J, *et al.* Temporal changes in the gut microbiota in farmed Atlantic cod (*Gadus morhua*) outweigh the response to diet supplementation with macroalgae [J]. *Animal Microbiome*, 2021, **3**(1): 7.
- [46] Cerezo I M, Fumanal M, Tapia-Paniagua S T, *et al.* Solea senegalensis bacterial intestinal microbiota is affected by low dietary inclusion of *Ulva ohnoi* [J]. *Frontiers in Microbiology*, 2022(12): 801744.
- [47] Nag D, Farr D A, Walton M G, *et al.* Zebrafish models for pathogenic vibrios [J]. *Journal of Bacteriology*, 2020, **202**(24): e00165-20.
- [48] AftabUddin S, Siddique M A M, Habib A, *et al.* Effects of seaweeds extract on growth, survival, antibacterial activities, and immune responses of *Penaeus monodon* against *Vibrio parahaemolyticus* [J]. *Italian Journal of Animal Science*, 2021, **20**(1): 243-255.
- [49] Bakky M A H, Tran N T, Zhang M, *et al.* In vitro fermentation of *Gracilaria lemaneiformis* and its sulfated polysaccharides by rabbitfish gut microbes [J]. *International Journal of Biological Macromolecules*, 2023(246): 125561.
- [50] You L, Gong Y, Li L, *et al.* Beneficial effects of three brown seaweed polysaccharides on gut microbiota and their structural characteristics: an overview [J]. *International Journal of Food Science & Technology*, 2020, **55**(3): 1199-1206.
- [51] Xie X, Chen C, Fu X. Modulation effects of *Sargassum pallidum* extract on hyperglycemia and hyperlipidemia in type 2 diabetic mice [J]. *Foods*, 2023, **12**(24): 4409.
- [52] Shi S, Nuccio E E, Shi Z J, *et al.* The interconnected rhizosphere: High network complexity dominates rhizosphere assemblages [J]. *Ecology Letters*, 2016, **19**(8): 926-936.
- [53] Boulangé C L, Neves A L, Chilloux J, *et al.* Impact of the gut microbiota on inflammation, obesity, and metabolic disease [J]. *Genome Medicine*, 2016, **8**(1): 42.
- [54] Dai W, Sheng Z, Chen J, *et al.* Shrimp disease progression increases the gut bacterial network complexity and abundances of keystone taxa [J]. *Aquaculture*, 2020(517): 734802.
- [55] Liu H, Yang X, Yang W, *et al.* Gut microbiota of freshwater gastropod (*Bellamya aeruginosa*) assist the adaptation of host to toxic cyanobacterial stress [J]. *Toxins*, 2023, **15**(4): 252.
- [56] Coyte K Z, Schluter J, Foster K R. The ecology of the microbiome: networks, competition, and stability [J]. *Science*, 2015, **350**(6261): 663-666.
- [57] Yuan M M, Guo X, Wu L, *et al.* Climate warming enhances microbial network complexity and stability [J]. *Nature Climate Change*, 2021(11): 343-348.
- [58] Han R, Wang L, Zhao Z, *et al.* Polysaccharide from *Gracilaria lemaneiformis* prevents colitis in Balb/c mice via enhancing intestinal barrier function and attenuating intestinal inflammation [J]. *Food Hydrocolloids*, 2020(109): 106048.
- [59] Ye C T, Wei X, Zhang Y. Current status of research on liver innate immunity [J]. *Journal of Clinical Hepatology*, 2014, **30**(9): 846-850. [叶传涛, 魏欣, 张颖. 肝脏固有免疫系统研究现状 [J]. *临床肝胆病杂志*, 2014, **30**(9): 846-850.]
- [60] Lv L G. Preliminary verification of C5/C5a/C5aR signaling pathway and some related factors in grass carp [D]. Changsha: Hunan Agricultural University, 2020: 26. [吕丽刚. 草鱼C5/C5a/C5aR信号通路及部分相关因子的初步验证 [D]. 长沙: 湖南农业大学, 2020: 26.]
- [61] Su H. Pro-inflammatory regulatory mechanism of

- C5a/C5aR pathway in response to inflammatory response to GCRV infection in grass carp [D]. Changsha: Hunan Agricultural University, 2022: 17-22. [苏杭. 草鱼 C5a/C5aR 通路应对 GCRV 感染炎症反应的促炎调控机制 [D]. 长沙: 湖南农业大学, 2022: 17-22.]
- [62] Kaufmann S H E. Immunology's coming of age [J]. *Frontiers in immunology*, 2019(10): 684.
- [63] Colley C S, Popovic B, Sridharan S, *et al.* Structure and characterization of a high affinity C5a monoclonal antibody that blocks binding to C5aR1 and C5aR2 receptors [J]. *mAbs*, 2018, **10**(1): 104-117.
- [64] Cyprian F S, Suleman M, Abdelhafez I, *et al.* Complement C5a and clinical markers as predictors of COVID-19 disease severity and mortality in a multi-ethnic population [J]. *Frontiers in Immunology*, 2021(12): 707159.
- [65] Köhl J, Baelder R, Lewkowich I P, *et al.* A regulatory role for the C5a anaphylatoxin in type 2 immunity in asthma [J]. *The Journal of Clinical Investigation*, 2006, **116**(3): 783-796.
- [66] Lopez-Santamarina A, Sinisterra-Loaiza L, Mondragón-Portocarrero A, *et al.* Potential prebiotic effect of two Atlantic whole brown seaweeds, *Saccharina japonica* and *Undaria pinnatifida*, using *in vitro* simulation of distal colonic fermentation [J]. *Frontiers in Nutrition*, 2023(10): 1170392.
- [67] Long X, Hu X, Liu S, *et al.* Insights on preparation, structure and activities of *Gracilaria lemaneiformis* polysaccharide [J]. *Food Chemistry: X*, 2021(12): 100153.
- [68] Nielsen B V, Maneein S, Al Farid M M, *et al.* The effects of halogenated compounds on the anaerobic digestion of macroalgae [J]. *Fermentation*, 2020, **6**(3): 85.
- [69] Lu S Y, Liu Y, Tang S, *et al.* *Gracilaria lemaneiformis* polysaccharides alleviate colitis by modulating the gut microbiota and intestinal barrier in mice [J]. *Food Chemistry: X*, 2022(13): 100197.
- [70] Wang A R, Ran C, Ringø E, *et al.* Progress in fish gastrointestinal microbiota research [J]. *Reviews in Aquaculture*, 2018, **10**(3): 626-640.
- [71] Takeuchi T, Nakanishi Y, Ohno H. Microbial metabolites and gut immunology [J]. *Annual Review of Immunology*, 2024, **42**(1): 153-178.
- [72] Yoo J Y, Groer M, Dutra S V O, *et al.* Gut microbiota and immune system interactions [J]. *Microorganisms*, 2020, **8**(10): 1587.
- [73] Wang X, Luo H, Zheng Y, *et al.* Effects of poultry by-product meal replacing fish meal on growth performance, feed utilization, intestinal morphology and microbiota communities in juvenile large yellow croaker (*Larimichthys crocea*) [J]. *Aquaculture Reports*, 2023(30): 101547.
- [74] Zhang X, Akhtar M, Chen Y, *et al.* Chicken jejunal microbiota improves growth performance by mitigating intestinal inflammation [J]. *Microbiome*, 2022, **10**(1): 107.
- [75] Liao X L, Chen Z F, Liu Q Y, *et al.* Tissue accumulation and biotransformation of 6PPD-quinone in adult zebrafish and its effects on the intestinal microbial community [J]. *Environmental Science & Technology*, 2024, **58**(23): 10275-10286.
- [76] Lan K P, Wu G D, Wang J, *et al.* Effects of dietary supplementation of inulin on survival, growth and intestinal microbiota of juvenile golden pompano (*Trachinotus ovatus*) [J]. *South China Fisheries Science*, 2022, **18**(5): 55-65. [兰鲲鹏, 吴光德, 王珺, 等. 饲料中添加菊粉对卵形鲳鲹幼鱼存活、生长和肠道菌群的影响 [J]. *南方水产科学*, 2022, **18**(5): 55-65.]
- [77] Ikeda-Ohtsubo W, López Nadal A, Zaccaria E, *et al.* Intestinal microbiota and immune modulation in zebrafish by fucoidan from Okinawa mozuku (*Cladosiphon okamuranus*) [J]. *Frontiers in Nutrition*, 2020(7): 67.

MACROALGAL SUPPLEMENTED DIET ON GUT MICROBIOTA AND IMMUNITY OF GRASS CARP DURING GCRV INFECTION

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Abstract: The grass carp (*Ctenopharyngodon idella*) is one of the major freshwater aquaculture species in China, contributing significantly to the aquaculture sector with a national production nearing 6 million tons in 2023. However, viral hemorrhagic disease caused by grass carp reovirus (GCRV) poses a significant disease affecting grass carp farming, often resulting to substantial economic losses in the industry. Macroalgae, characterized by their diversity, wide oceanic distribution, and high productivity, harbor a wealth of natural bioactive compounds including algal polysaccharides, alginates, algal polyphenols, and dietary fiber. These substances can regulate the nutrients metabolism and absorption in animals and exhibit proven antiviral and antioxidant activities. Currently, macroalgae are used as natural feed components in aquaculture to bolster fish disease resistance. However, the potential impact of incorporating macroalgae on GCRV infection in grass carp via the modulation of gut microbiota and host immunity remains uncertain. In this study, a dried powder comprising *Sargassum hemiphyllum*, *Asparagopsis taxiformis*, and *Gracilaria lemaneiformis* was added to grass carp feed for two months, followed by intraperitoneal GCRV infection. High-throughput 16S rRNA gene sequencing was used to analyze the gut microbiota, with subsequent quality control and assembly of raw sequencing reads to obtain high-quality paired-end sequences. Chimerism sequences was identified and removed, while sequences exhibiting a similarity above a defined threshold of 97 were clustered into Operational Taxonomic Units (OTUs). These OTUs were annotated by using RDP classifier based on Silva 16S rRNA database. Subsequently, species annotation was conducted, resulting in the acquisition of raw abundance data. Enzyme-linked immunosorbent assay (ELISA) was used to detect the expression levels of complement component 5 (C5), C5a, and C5a receptor (C5aR) in the serum to assess the host immune response. The results showed that macroalgae supplementation significantly increased the Shannon and Simpson indices of gut microbiota in grass carp. NMDS, Venn diagram, and heatmap analysis indicated a significant restructuring of the gut microbiota following macroalgae supplementation. This restructuring led to an increased presence of distinct bacterial species primarily associated with dietary fiber fermentation and carbohydrate digestion. Furthermore, it contributed to a delayed decline in gut microbiota diversity observed on the initial day post-GCRV infection. Differential analysis, based on Bray-Curtis distance, was performed for the gut microbiota before GCRV infection (day 0, G0) and on days 4 and 7 post-infection (G4 and G7). The results revealed a significant alteration in the gut microbiota structure of the control group between days 4 and 7 post-GCRV infection, while no significant changes were observed in the macroalgae supplementation group. Specifically, the relative abundance of *Vibrio* in the intestinal tract reduced significantly in the groups fed with *S. hemiphyllum* and *G. lemaneiformis*. In the *S. hemiphyllum* group, the relative abundance of *Cetobacterium* decreased, while that of *Bacteroides* increased. Co-occurrence network analysis of the gut microbiota from the infected grass carp indicated that macroalgae enhanced the network complexity of the microbiota, alleviating the impact of GCRV on their gut microbiota. Analysis of complement expression in the serum of infected grass carp revealed that *S. hemiphyllum* upregulated C5a expression on day 1 post-infection, while *G. lemaneiformis* downregulated C5a expression. Spearman correlation analysis suggested that after GCRV infection, macroalgae might influence the expression of C5, C5a, and its receptor (C5aR) by regulating beneficial and pathogenic bacteria in the intestine. In conclusion, the addition of macroalgae alleviates the impact of GCRV on the gut microbiota of grass carp, with *G. lemaneiformis* contributing to the reduction of inflammatory responses. These findings could pave the way for novel strategies in the prevention and management of hemorrhagic disease in grass carp, offering significant practical implications for the aquaculture industry.

Key words: Grass carp hemorrhagic disease; Macroalgae; GCRV; Gut microbiota; Complement