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碳酸氢钠对慢性高温胁迫下大口黑鲈生长性能、血液生化和肠道损伤的影响

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SODIUM BICARBONATE ON GROWTH PERFORMANCE, BLOOD BIOCHEMICAL PARAMERERS, AND INTESTINAL DAMAGE OF LARGEMOUTH BASS (*MICROPTERUS SALMOIDES*) UNDER CHRONIC HEAT STRESS

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摘要: 为了探究碳酸氢钠($NaHCO_3$)对慢性高温胁迫下大口黑鲈(*Micropterus salmoides*)肠道损伤的影响, 实验以大口黑鲈幼鱼(20.26±0.08) g为研究对象, 分别投喂含有或不含碳酸氢钠($NaHCO_3$, 5 g/kg)的饲料8周。结果表明, 在慢性高温胁迫下, $NaHCO_3$组特定生长率与对照组相比无显著差异, 但饲料效率、蛋白质沉积率和蛋白质效率, 以及肠道淀粉酶和胰蛋白酶活性显著提高。$NaHCO_3$的添加显著提高了肠道总抗氧化能力(T-AOC)、过氧化氢酶(CAT)、超氧化物歧化酶(SOD)和谷胱甘肽过氧化物酶(GPx)活性, 并上调了*cat*和*gpx1a*基因的表达, 下调了*keap1*的基因表达。同时, $NaHCO_3$显著下调了促炎症细胞因子(*il-1β*、*tnfα*、*il15*)的表达, 上调了抗炎细胞因子*il10*的表达。此外, $NaHCO_3$组大口黑鲈肠道的*hsp90*、*hsp70*和*hsp60*基因的表达均显著低于对照组。肠道组织学分析表明, 高温胁迫引起了大口黑鲈肠道损伤, 饲料中添加$NaHCO_3$显著改善了肠道组织形态, 并上调了肠道*zo1*、*jam*、*occludin*、*muc2*的表达。综上所述, 饲料中添加5 g/kg $NaHCO_3$通过提高肠道抗氧化能力、改善肠道炎症和肠道屏障功能来有效缓解慢性高温胁迫诱导的大口黑鲈肠道损伤。研究为改善鱼类在高温胁迫下的肠道健康提供了新的视角。

关键词: 碳酸氢钠; 高温胁迫; 生长性能; 肠道健康; 大口黑鲈

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在水产养殖过程中, 养殖水体温度被认为是最关键的环境因素之一。随着全球气候变暖的加剧, 养殖水温逐渐升高, 鱼类的热应激程度也随之增加[1]。近年来, 中国多个省份在夏季6—8月期间气温超过40℃, 地面温度甚至超过45℃[2]。持续的极端高温使得水温超过了鱼类的耐热限度, 影响其生长与健康[3]。以华南地区为例, 夏季室外池塘养殖水温常常升高至35℃或更高, 导致水产养殖动物摄食量显著下降, 生长速度减缓, 抗病能力降低, 进而严重影响经济效益[4, 5]。

大口黑鲈(*Micropterus salmoides*), 鲈形目棘臀鱼科黑鲈属, 1983年由北美引进, 因其肉质鲜嫩、营养丰富、生长迅速且易于捕捞, 具有较高的经济价值, 是优良的淡水养殖品种之一[6]。在高温季节, 特别是水温超过28℃时, 大口黑鲈容易出现热应激反应, 免疫力下降, 易感染疾病。同时, 高温导致溶氧不足和水质恶化问题进一步加剧, 进而引发病害暴发和经济损失[7]。研究表明, 在高温环境下, 鱼类的肠道结构容易受到破坏, 进而导致肠道菌群失衡, 引发严重的生理功能障碍[8]。同时高温胁迫会损害大口黑鲈的肠道健康, 导致其营养吸收能力下降[9]。慢性高温应激是诱发鱼类肠炎的主要因素之一, 高温使得大口黑鲈小肠上皮细胞的完整性易受损, 导致肠道黏膜通透性增加, 致病菌容易侵入, 引发炎症反应[10, 11]。

营养调控是指通过调整饲料成分等促进动物

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营养吸收、增强免疫力等过程[12]。碳酸氢钠($NaHCO_3$)作为一种无机盐, 具有调节促生长、抗氧化、促进消化、增强免疫力和缓解热应激等多重功能[13]。研究表明, $NaHCO_3$通过改善水质口感和促进饮水量, 有效提升热应激条件下公鸡(*Gallus*)的生长性能和饲料利用效率[14]。同时, 在肉鸡(*Gallus domesticus*)中添加$NaHCO_3$显著提高了日增重, 并缓解了慢性高温胁迫引起的呼吸性碱中毒[15]。此外, 作为一种弱碱, $NaHCO_3$可提高机体血液pH, 进而中和代谢物中的酸性物质[16]。$NaHCO_3$显著改善了羔羊(*Agnus dei*)和羊(*Capra hircus* L.)的生长性能, 并有效降低了瘤胃pH[17, 18]。在山羊中联合使用铬(1.5 mg/d)和$NaHCO_3$(15 g/kg)显著提高了生长性能, 并通过缓解热胁迫的负面影响, 改善了饲料利用率[19]。尽管$NaHCO_3$在多种动物中的应用已有一定研究, 但在鱼类中的相关研究仍较为有限。本文推测$NaHCO_3$可能提高大口黑鲈耐高温能力。为了验证我们的假设, 我们探讨了在慢性高温胁迫下, 添加5 g/kg $NaHCO_3$对大口黑鲈生长、生化指标和肠道健康的影响, 以期为缓解鱼类热应激提供理论依据。

1 材料与方法

1.1 实验饲料

本实验所用基础饲料以鱼粉、鸡肉粉和棉籽浓缩蛋白为主要蛋白源, 配制的粗蛋白含量为55%, 粗脂肪含量为8.5%。研究表明日粮中添加5 g/kg $NaHCO_3$显著缓解夏季热应激对肉鸡生长性能和肝脏健康的影响[20], 因此本实验在基础饲料中添加5 g/kg $NaHCO_3$(纯度≥98%, 上海源叶生物科技有限公司, 中国上海)制备成实验组饲料。所有原料经过超微粉碎机粉碎, 过80目筛网, 随后用木薯淀粉逐步稀释, 并与其他原料充分混合。加水搅拌均匀后, 使用双螺杆挤压机(SLP−45, 上海渔业机械设备研究所, 中国)制备粒径约为4 mm的膨化颗粒饲料, 70℃烘干后放置4℃中保存备用。饲料配方见表 1。

1.2 实验鱼及养殖条件

实验所用的大口黑鲈购自湖北正好水产养殖公司(中国湖北)。养殖实验在湖北省石首市老河长江四大家鱼原种场的池塘网箱(2 m×2 m×2 m)中进行。实验鱼在网箱内暂养, 使用基础饲料饲喂2周。实验鱼在正式实验前饥饿24h。挑选健康、规格均匀的420尾幼鱼[初始均重: (20.26±0.08) g], 称重后随机放入6个网箱中。实验期间, 每日饱食投喂2次(6:30、17:30)。养殖实验持续8周。实验水温为(34.9±0.3)℃(图 1), 溶解氧>6 mg/L, 氨氮含量为0.20—0.34 mg/L, 亚硝酸盐含量为0.009—0.014 mg/L, pH为6.8—7.2。

1.3 样品采集

在实验开始前, 选取规格相近的3组实验鱼作为初样, 保存在−20℃冰箱中备用。在养殖实验结束后, 使用MS-222 (60 mg/L, Sigma, 美国)对实验鱼进行麻醉, 并进行称重和计数。每个网箱随机抽取2尾鱼作为末样, 用于基本组分的测定。同时, 随机

表 1 实验饲料配方及化学组成(%干物质)

Tab. 1 Diet formulation and chemical composition (% dry matter)

原料Ingredient	饲料Diet	
	CON	$NaHCO_3$
鱼粉Fish meal	42.00	42.00
鸡肉粉Chicken powder	12.00	12.00
棉籽浓缩蛋白Cottonseed protein concentrate	9.00	9.00
谷朊粉Wheat flour	5.00	5.00
豆粕Soybean meal	7.00	7.00
木薯淀粉Tapioca flour	12.00	12.00
鱼油Fish oil	2.00	2.00
豆油Soybean oil	2.00	2.00
多维多矿预混物Vitamin and mineral premix[1]	2.00	2.00
磷酸二氢钙$Ca(H_2PO_4)_2$	1.00	1.00
氯化胆碱Choline chloride	0.30	0.30
DL-蛋氨酸DL-Methionine	0.21	0.21
L-赖氨酸盐酸盐L-Lysine hydrochloride (98%)	0.36	0.36
碳酸氢钠$NaHCO_3$	0.00	0.50
纤维素Cellulose	5.13	4.63
化学组成Chemical composition (%)		
水分Moisture	7.12	6.97
粗蛋白Crude protein	54.62	55.07
粗脂肪Crude lipid	8.52	8.38

注: [1]多维多矿预混物: 七水硫酸镁, 205.39 mg/kg; 七水硫酸铁, 198.57 mg/kg; 七水硫酸锰, 50.36 mg/kg; 碘化钾, 0.07 mg/kg; 五水硫酸铜, 98.30 mg/kg; 七水硫酸锌, 442.46 mg/kg; 亚硒酸钠, 0.66 mg/kg; 七水硫酸钴, 1.67 mg/kg; 维生素A, 1800 IU/kg; 维生素D3, 390 IU/kg; 维生素E, 30 mg/kg; 维生素K3, 2.8 mg/kg; 维生素B1, 6 mg/kg; 维生素B2, 6 mg/kg; 维生素B6, 6 mg/kg; 维生素B12, 0.04 mg/kg; 维生素C, 70 mg/kg; 泛酸钙, 15 mg/kg; 烟酰胺, 45 mg/kg; 叶酸, 2 mg/kg; D-生物素, 0.05 mg/kg; 肌醇, 40 mg/kg; 玉米淀粉, 4500 mg/kg。购自广东联鲲集团有限公司

Note: [1]Vitamin and mineral premix: $MgSO_4 \cdot 7H_2O$, 205.39 mg/kg; $FeSO_4 \cdot 7H_2O$, 198.57 mg/kg; $MnSO_4 \cdot 7H_2O$, 50.36 mg/kg; KI, 0.07 mg/kg; $CuSO_4 \cdot 5H_2O$, 98.30 mg/kg; $ZnSO_4 \cdot 7H_2O$, 442.46 mg/kg; Na_2SeO_3, 0.66 mg/kg; $CoSO_4 \cdot 7H_2O$, 1.67 mg/kg; Vitamin A, 1800 IU/kg; Vitamin D3, 390 IU/kg; Vitamin E, 30 mg/kg; Vitamin K3, 2.8 mg/kg; Vitamin B1, 6 mg/kg; Vitamin B2, 6 mg/kg; Vitamin B6, 6 mg/kg; Vitamin B12, 0.04 mg/kg; Vitamin C, 70 mg/kg; Calcium pantothenate, 15 mg/kg; Nicotinamide, 45 mg/kg; Folic acid, 2 mg/kg; D-biotin, 0.05 mg/kg; Myo-inositol, 40 mg/kg; Corn starch, 4500 mg/kg. Purchased from Guangdong Liankun Group Co., Ltd (Guangdong, China)

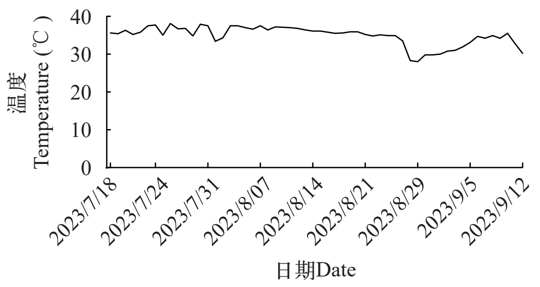

图 1　实验间日均水温变化趋势

Fig. 1　The mean daily water temperature during the experiment

抽取2尾鱼, 使用肝素钠抗凝剂浸润过的1 mL无菌注射器采集尾部静脉血样, 4℃下离心分离血浆, 并在冰浴条件下快速取出肠道样品。血浆和肠道样品均存放于−80℃保存备用。部分肠道组织用4%多聚甲醛固定液固定, 通过组织学分析肠道形态[21]。

1.4　样品生化分析

实验饲料和鱼体基本组分(粗蛋白、粗脂肪、水分和灰分)含量按照AOAC[22]标准方法进行测定。采用全自动生化分析仪(BS-460, 深圳迈瑞生物医疗电子股份有限公司, 深圳)测定血浆总蛋白(Total protein, P/N:105-015578-00)、白蛋白(Albumin, P/N:105-000450-00)、碱性磷酸酶(Alkaline phosphatase, P/N: 105-000444-00)和葡萄糖(Glucose, P/N: 105-000949-00)[23]。

迅速剪取约50 mg中肠组织, 置入1.5 mL离心管底部, 按1∶9的比例加入0.68%的无菌生理盐水, 在低温条件下用高速组织匀浆机匀浆。随后在4℃、3000 r/min条件下离心10 min, 取上清制成10%的肠道组织匀浆液, 使用考马斯亮蓝试剂盒(碧云天生物技术研究所, P0006)测定蛋白质含量。同时, 使用南京建成生物工程研究所的试剂盒检测总抗氧化能力(T-AOC, A015-2)、谷胱甘肽过氧化物酶(GPx, A005-1-2)、过氧化氢酶(CAT, A007-1-1)、超氧化物歧化酶(SOD, A001-3-2)活性及丙二醛(MDA, A003-1-2)含量, 检测肠道胰蛋白酶(A080-2-2)、脂肪酶(A054-2-1)和α-淀粉酶(C016-1-2)活性。

1.5　基因表达分析

根据Wu等[24]的方法, 使用Trizol试剂(Invitrogen, 美国)提取肠道组织RNA, 利用M-MLV逆转录酶(M-MLV)将RNA反转录为cDNA。使用LightCycle 480 II系统(Roche, Basel, Switzerland)和2× TSINGKE® Master SYBR Green I qPCR Mix-UDG (Without ROX)荧光染料进行实时荧光定量PCR检测。*β-actin*为内参基因, 根据引物扩增效率计算目标基因的相对表达水平[25]。相关基因的引物序列见表 2。

1.6　数据统计分析

所有数据均以平均数±标准误表示(n=6)。数据的正态分布情况通过SPSS 19.0软件中的Shapiro-Wilk检验进行评估。随后, 采用独立样本t检验(Independent sample t-test)比较对照组(CON)和碳酸氢钠($NaHCO_3$)组的差异。P<0.05被认为具有显著性差异。

2　结果

2.1　生长与鱼体组成

如表 3所示, 在高温胁迫下, 饲料中添加$NaHCO_3$显著提高了大口黑鲈的饲料效率(FE)、蛋白质效率(PER)和蛋白质沉积率(PRE; P<0.05)。与对照组相比, $NaHCO_3$组的终末体重(FBW)、摄食量(FI)、增重率(WGR)、特定生长率(SGR)和存活率(SR)无显著差异(P>0.05)。鱼体组分分析结果显示, $NaHCO_3$组的全鱼粗脂肪含量显著低于对照组(P<0.05)。

2.2　血浆生化指标

大口黑鲈的血浆生化指标如图 2所示。在高温胁迫下, 饲料中添加$NaHCO_3$显著提高了大口黑鲈血浆中碱性磷酸酶和总蛋白的含量(P<0.05), 对白蛋白和葡萄糖水平无显著影响(P>0.05)。

2.3　肠道抗氧化及消化酶指标

如表 4所示, 与对照组相比, 饲料中添加$NaHCO_3$显著提高了大口黑鲈肠道中的总抗氧化能力(T-AOC)、过氧化氢酶(CAT)、超氧化物歧化酶(SOD)和谷胱甘肽过氧化物酶(GPx)活性(P<0.05), 对丙二醛(MDA)含量无显著影响(P>0.05)。在肠道消化酶活性方面, $NaHCO_3$显著提高了肠道淀粉酶和胰蛋白酶的活性(P<0.05), 但对脂肪酶活性无显著影响(P>0.05)。

2.4　肠道抗氧化、炎症和热休克蛋白家族相关基因表达

在高温胁迫条件下, 与对照组相比, 饲料中添加$NaHCO_3$显著降低了肠道热休克蛋白(*hsp90*、*hsp70*、*hsp60*)的基因表达(P<0.05), 而对*hsp30*和*hsp27*的转录水平无显著影响(P>0.05; 图 3)。如图 4所示, $NaHCO_3$处理显著上调了肠道抗氧化相关基因(*cat*、*gpx1a*)的表达, 并显著下调了*keap1*的基因表达(P<0.05), 但是不影响*nrf2*和*sod1*的基因表达(P>0.05)。在慢性高温胁迫条件下, 添加$NaHCO_3$显著下调了促炎症细胞因子(*il-1β*、*tnfα*和*il15*)的基因表达, 同时上调了抗炎细胞因子*il10*的表达(P<0.05; 图 5)。

2.5　肠道组织学及紧密连接相关基因的表达

$NaHCO_3$添加对慢性高温胁迫下大口黑鲈肠道

组织学的影响如图 6所示。HE染色结果显示, 在慢性高温胁迫8周后, 对照组大口黑鲈肠道绒毛明显缩短且排列稀疏, 部分黏膜细胞脱落; 而$NaHCO_3$组肠道形态较为完整, 结构清晰, 肠绒毛排列紧密。两组间肌层厚度差异不显著, 但$NaHCO_3$显著提高了大口黑鲈肠道绒毛长度和杯状细胞数量。同时,

表 2 本实验所用引物序列表

Tab. 2 Primers used in the present experiment

基因名Gene	引物序列Primer sequence(5′—3′)	登录号Accession number
β-actin	CTGTGGTGGTGAATGAGTAGCC CATCCTCCGTTTGGACTTGG	PRJNA725023
Heat shock protein 90 (*hsp90*)	ACCCCTCTCTCCCTGGGAATT GCACGCTCACCCTCATAAACC	XM_038708948.1
Heat shock protein 70 (*hsp70*)	GTCCTACGCCTTCAACACGA GCTGATGGTCTCGTCACACT	XM_038708948.1
Heat shock protein 60 (*hsp60*)	AAGCAGGTGAGGCCTGTGTG AGCATGAGGGCACGAGCATC	XM_038721348.1
Heat shock protein 30 (*hsp30*)	CTACGTACAGGGTTGCGGGG ATGGACTCAGCTACATGTTGTTCT	XM_038732984.1
Heat shock protein 27 (*hsp27*)	ATCGGGCAAATCGCCTTCA AGACCACCGTGTGATTGCT	XM_038709260.1
Interleukin 1β (*il-1β*)	CGTGACTGACAGCAAAAAGAGG GATGCCCAGAGCCACAGTTC	XM_038696252.1
Interleukin 15 (*il15*)	GTATGCTGCTTCTGTGCCTGG AGCGTCAGATTTCTCAATGGTGT	XM_038713163.1
Interleukin 10 (*il10*)	CGATTCTGCCAACAGCCTTG GCTCGTCGAAGATCTGCTGT	XM_038696252.1
Tumor necrosis factor α (*tnfα*)	CTTCGTCTACAGCCAGGCATCG TTTGGCACACCGACCTCACC	XM_038710731.1
Transforming growth factor β (*tgfβ*)	GCTCAAAGAGAGCGAGGATG TCCTCTACCATTCGCAATCC	XM_038693206.1
Nuclear factor (*erythroid-derived 2*)*-like 2* (*nrf2*)	TCACCAAAGACAAGCGTAA CAGGCAGATTGATAATCATAGA	XM_038720536.1
Kelch-like ECH-associated protein-1 (*keap1*)	GATAGACAGCGTGGTCAAGGC TGAAGAACTCCTCCTGGGTCG	MW465394
Catalase (*cat*)	GTTCCCGTCCTTCATCCACT CAGGCTCCAGAAGTCCCACA	MK614708.1
Superoxide dismutase 1 (*sod1*)	GCGTGGGTAGATGGTTT AGGGTTGATGGGCAGTA	XM_038713969.1
Glutathione peroxidase 1a (*gpx1a*)	CCCTGCAATCAGTTTGGACA TTGGTTCAAAGCCATTCCCT	XM_038697220.1
Zonula occludens-1 (*zo-1*)	ATCTCAGCAGGGATTCGACG CTTTTGCGCTGGCGTTGG	XM_038701018.1
Mucin 2 (*muc2*)	AAAATAAGGAGGGTTGGG GCTGTGCCGTGGTTCTAG	XM_038706114.1
Occludin	GATATGGTGGCAGCTACGGT TCCTACTGCGGACAGTGTTG	XM_038715419.1
Claudin	CCAGGGAAGGGGAGCAATG GCTCTTTGAACCAGTGCGAC	XM_038713307.1
Junctional adhesion molecule (*jam*)	TAGGTTTGCTGCTCTTTGGTCT GTCTGAATCCGTTAGCCTCATC	NM_001004667

$NaHCO_3$处理显著提高了慢性高温胁迫条件下大口黑鲈肠道组织中紧密连接相关基因*zo1*、*jam*、*occludin*和*muc2*的表达水平(*P*<0.05; 图 7)。

表 3　饲料中添加$NaHCO_3$对慢性高温胁迫下大口黑鲈生长、饲料利用和鱼体营养成分的影响

Tab. 3　Effects of dietary $NaHCO_3$ supplementation on growth, feed utilization, and fish nutritional composition of largemouth bass under chronic heat stress

指标Item	饲料Diet	
	CON	$NaHCO_3$
初始体重 IBW[1] (g)	20.25±0.11	20.17±0.17
终末体重 FBW[2] (g)	92.93±0.88	102.73±3.44
摄食量 FI[3] (g/fish)	93.40±2.76	93.01±3.17
增重率 WGR[4] (%)	358.79±6.79	409.13±14.40
饲料效率 FE[5] (%)	83.92±1.38[a]	86.27±1.16[b]
特定生长率 SGR[6] (%/d)	2.72±0.03	2.90±0.05
存活率 SR[7] (%)	86.19±0.48	87.14±3.78
蛋白质效率 PER[8]	1.41±0.03[a]	1.56±0.02[b]
蛋白质沉积率 PRE[9] (%)	20.66±1.33[a]	26.18±0.39[b]
初始鱼体营养成分 Initial fish nutritional composition		
粗蛋白 Crude protein (%)	8.10±0.14	8.10±0.14
粗脂肪 Crude lipid (%)	20.01±1.09	20.01±1.09
灰分 Ash (%)	16.56±0.52	16.56±0.52
水分 Moisture (%)	65.10±1.24	65.10±1.24
终末鱼体营养成分Final fish nutritional composition (%)		
粗蛋白 Crude protein	15.77±0.82	16.67±0.21
粗脂肪 Crude lipid	7.47±0.21[b]	6.76±0.18[a]
灰分 Ash	15.65±1.85	17.42±0.35
水分 Moisture	71.45±1.29	70.52±0.86

注: 表中数据表示为平均值±标准误(*n*=3), 同一行中不同数值的英文字母上标表示差异显著(*P*<0.05)。The data are expressed as means±SE (*n*=3), and the different superscript letters in the same row indicate significant differences (*P*<0.05); [1]初始体重 IBW (g): Initial body weight; [2]终末体重 FBW (g): Final body weight; [3]摄食量 FI (g/fish)=干物质摄食量/鱼数目; Feed intake (FI, g/fish)=dry feed intake/number of fish; [4]增重率WGR (%)=100×(终末体重–初始体重)/初始体重; Weight gain rate (WGR, %)=100×(FBW–IBW)/IBW; [5]饲料效率 FE(%)=(100×鱼体湿重增重量)/干物质摄食量; Feed efficiency (FE, %)=(100×fresh body weight gain)/dry feed intake; [6]特定增长率 SGR (%/d)=100×[ln(终末体重)–ln(初始体重)]/投喂天数; Specific grow rate (SGR, %/d)=100×[ln (final body weight)–ln (initial body weight)]/days; [7]存活率SR (%)=(存活鱼总数/起始鱼总数)×100; Survival rate (SR, %)=100×(final fish number/initial fish number); [8]蛋白质效率PER = (终末体重–初始体重)/蛋白摄入量; Protein efficiency ratio (PER)=(final body weight–initial body weight)/protein intake; [9]蛋白质沉积率PRE (%)=100×(末样体蛋白×终末体重–初样体蛋白×初始体重)/蛋白摄入量; Protein retention efficiency (PRE, %)=100×(final body protein×final body weight–initial body protein×initial body weight)/protein intake

3　讨论

3.1　$NaHCO_3$对慢性高温胁迫下大口黑鲈生长性能和血浆生化指标的影响

水温是水产养殖的重要因素, 高温会引起水产养殖动物的应激, 从而影响生长和免疫等。在中华

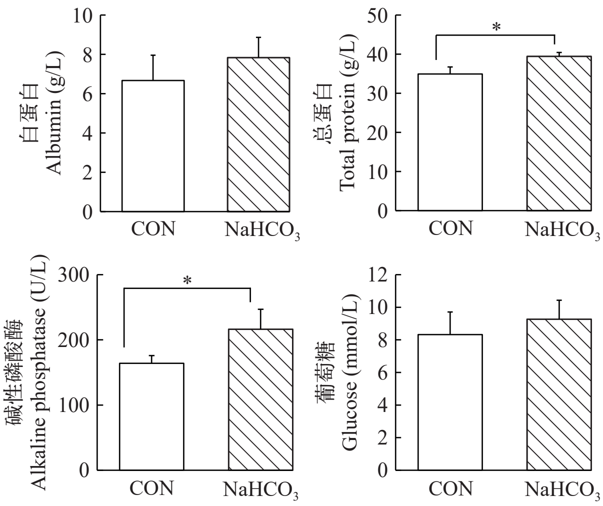

图 2　饲料中添加$NaHCO_3$对慢性高温胁迫下大口黑鲈血浆生化指标的影响

Fig. 2　Effects of dietary $NaHCO_3$ on serum biochemical indices in largemouth bass under chronic heat stress

柱状图上方的*或者**表示各处理间的差异, *表示显著性差异(*P*<0.05), **表示极显著差异(*P*<0.01); 结果用均值±标准误(*n*=6)表示

The * or ** at the top of the bar chart indicates significant differences between treatments; * indicates a significant difference (*P*<0.05), and ** indicates an extremely significant difference (*P*<0.01); The values are expressed as the mean±SEM (*n*=6)

表 4　饲料中添加$NaHCO_3$对慢性高温胁迫下大口黑鲈肠道抗氧化能力和消化酶活性的影响

Tab. 4　Effects of dietary $NaHCO_3$ on antioxidant enzyme activity and digestive enzymes in gut of largemouth bass under chronic heat stress

指标Item	CON	$NaHCO_3$
总抗氧化能力T-AOC (nmol/mg prot)	0.31±0.01[a]	0.38±0.02[b]
超氧化物歧化酶SOD (U/mg prot)	15.88±2.83[a]	31.32±2.85[b]
丙二醛MDA (nmol/mg prot)	2.62±0.15	2.31±0.40
过氧化氢酶CAT (U/mg prot)	13.50±1.84[a]	25.71±1.75[b]
谷胱甘肽过氧化物酶GPx (U/mg prot)	10.45±0.67[a]	27.69±2.30[b]
脂肪酶 (U/g prot)	1.73±0.17	2.65±0.41
α-淀粉酶 (U/mg prot)	0.88±0.08[a]	1.37±0.06[b]
胰蛋白酶 (U/mg prot)	2004.08±619.86[a]	6322.22±1502.28[b]

注: 同一行不同上标字母表示差异显著(*P*<0.05)

Note: Different superscript letters in the same row indicate significant differences (*P*<0.05)

绒螯蟹(*Eriocheir sinensis*)中, 高温应激增加了中华绒螯蟹摄食量, 并促进其生长[26]。饲料中添加鱼腥草可改善高温应激对大口黑鲈的影响, 提高其耐高温能力[5]。碳酸氢钠在畜禽养殖中广泛应用于缓解高温应激, 不仅能够改善畜禽的抗应激能力, 还能提高饲料利用率并加速营养物质的吸收[27]。饲料中添加$NaHCO_3$可缓解热应激对山羊的饲料消耗、消化能力、代谢和生理功能的不利影响, 从而提高雄鹿的生长性能和饲料利用[19]。在本实验中, 饲料

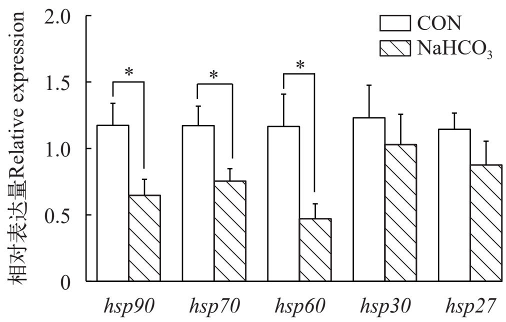

图 3 $NaHCO_3$添加对慢性高温胁迫下大口黑鲈肠道热休克蛋白相关基因表达的影响

Fig. 3 Effect of $NaHCO_3$ on heat shock protein-related gene expression in largemouth bass under chronic heat stress

柱状图上方的*或者**表示各处理间的差异, *表示显著性差异($P<0.05$), **表示极显著差异($P<0.01$); 结果用均值±标准误($n=6$)表示

The * or ** at the top of the bar chart indicates significant differences between treatments; * indicates a significant difference ($P<0.05$), and ** indicates an extremely significant difference ($P<0.01$); The values are expressed as the mean±SEM ($n=6$)

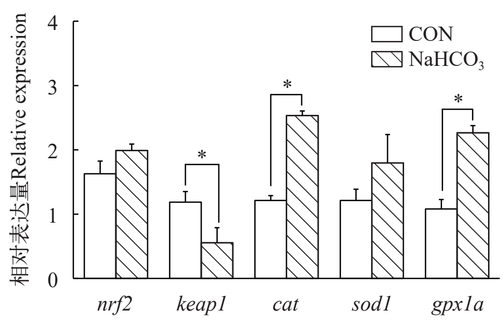

图 4 $NaHCO_3$添加对慢性高温胁迫下大口黑鲈肠道抗氧化相关基因表达的影响

Fig. 4 Effect of $NaHCO_3$ on antioxidant-related gene expression in largemouth bass under chronic heat stress

柱状图上方的*或者**表示各处理间的差异, *表示显著性差异($P<0.05$), **表示极显著差异($P<0.01$); 结果用均值±标准误($n=6$)表示

The * or ** at the top of the bar chart indicates significant differences between treatments; * indicates a significant difference ($P<0.05$), and ** indicates an extremely significant difference ($P<0.01$); The values are expressed as the mean±SEM ($n=6$)

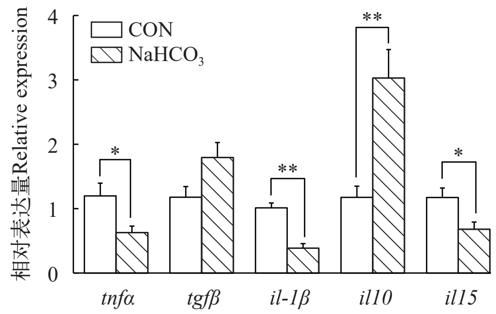

图 5 $NaHCO_3$添加对慢性高温胁迫下大口黑鲈肠道炎症相关基因表达的影响

Fig. 5 Effect of $NaHCO_3$ on inflammation-related gene expression in largemouth bass under chronic heat stress

柱状图上方的*或者**表示各处理间的差异, *表示显著性差异($P<0.05$), **表示极显著差异($P<0.01$); 结果用均值±标准误($n=6$)表示

The * or ** at the top of the bar chart indicates significant differences between treatments; * indicates a significant difference ($P<0.05$), and ** indicates an extremely significant difference ($P<0.01$); The values are expressed as the mean±SEM ($n=6$)

中添加$NaHCO_3$对高温胁迫下的大口黑鲈的生长性能无显著影响, 但提高了饲料利用率。在合方鲫(*Carassius auratus*)中, 其增重率和特定生长率随着$NaHCO_3$浓度的增加反而下降[28]。因此, $NaHCO_3$的作用可能与品种、胁迫时间、应激强度和养殖环境有关。

血浆总蛋白含量常被用来反映鱼类的免疫水平[29]。结果显示, 饲料中添加$NaHCO_3$显著提高了大口黑鲈血浆中总蛋白含量, 对白蛋白无显著影响, 这与之前的研究一致[30]。血浆碱性磷酸酶是一种在鱼类等生物体内广泛存在的酶, 其活性通常与机体的营养状况、免疫及代谢状态等密切相关[31]。碱性磷酸酶活性升高不仅可以增强机体的免疫力, 还可以通过调节机体的能量代谢来适应高温胁迫。在本实验中, $NaHCO_3$组的血浆碱性磷酸酶活性显著高于对照组。这可能是因为$NaHCO_3$能够中和部分酸性代谢产物, 维持机体酸碱平衡, 从而减轻热应激对机体的损伤。

3.2 $NaHCO_3$对慢性高温胁迫下大口黑鲈肠道抗氧化能力的影响

氧化应激主要是活性氧(ROS)和抗氧化能力之间的不平衡引起的, 而热应激是导致活性代谢物清除失衡的主要应激源之一[32]。总抗氧化能力(T-AOC)、超氧化物歧化酶(SOD)、过氧化氢酶(CAT)和谷胱甘肽过氧化物酶(GSH-Px)是机体抗氧化防御系统的重要指标, 能有效清除体内ROS, 减轻脂质过氧化反应, 抵御氧化应激[33]。与肉鸡中的研究结果一致[27], $NaHCO_3$添加后通过增加大口黑鲈肠道抗氧

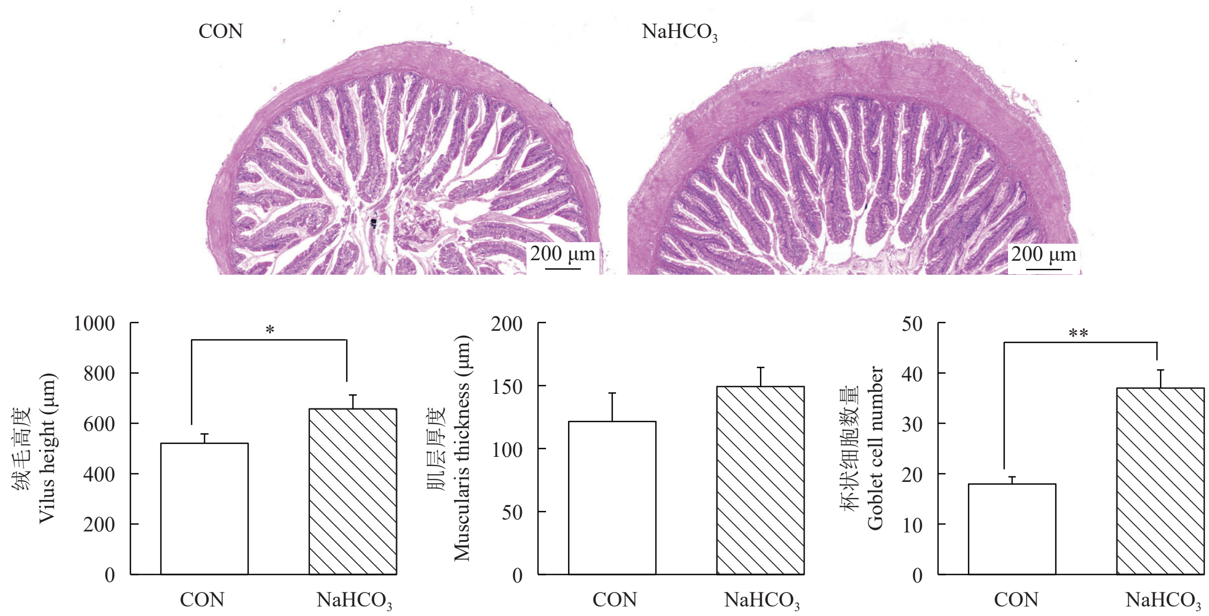

图 6　$NaHCO_3$添加对慢性高温胁迫下大口黑鲈肠道组织学的影响

Fig. 6　Effects of $NaHCO_3$ on gut histology in largemouth bass under chronic heat stress

柱状图上方的*或者**表示各处理间的差异, *表示显著性差异(P<0.05), **表示极显著差异(P<0.01); 结果用均值±标准误(n=6)表示

The * or ** at the top of the bar chart indicates significant differences between treatments; * indicates a significant difference (P<0.05), and ** indicates an extremely significant difference (P<0.01); The values are expressed as the mean±SEM (n=6)

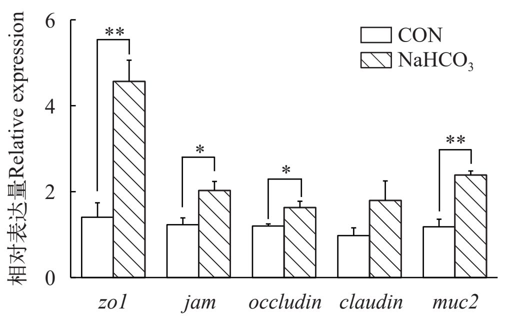

图 7　$NaHCO_3$添加对慢性高温胁迫下大口黑鲈肠道紧密连接相关基因表达的影响

Fig. 7　Effects of $NaHCO_3$ on intestinal tight junction-related gene expression in largemouth bass under chronic heat stress

柱状图上方的*或者**表示各处理间的差异, *表示显著性差异(P<0.05), **表示极显著差异(P<0.01); 结果用均值±标准误(n=6)表示

The * or ** at the top of the bar chart indicates significant differences between treatments; * indicates a significant difference (P<0.05), and ** indicates an extremely significant difference (P<0.01); The values are expressed as the mean±SEM (n=6)

化酶活性来减轻慢性高温胁迫引起的氧化应激。同样, 在H9C2细胞中, 维生素C和$NaHCO_3$联合使用显著提高了热应激抑制的抗氧化酶活性[13]。Nrf2作为一种核内转录因子, 通过调控一系列抗氧化相关基因的表达, 协同调控ROS引起的氧化应激[34]。本实验中$NaHCO_3$处理显著上调了大口黑鲈肠道抗氧化基因(*cat*、*gpx1a*)的表达, 并显著下调了*keap1*的基因表达。在杂交鲟(*Acipenser baerii* ♀×*A. schrenkii* ♂)[35]和梭鲈(*Sander lucioperca*)[33]中也观察到类似的结果。这些结果表明, $NaHCO_3$添加后通过增强大口黑鲈抗氧化能力来缓解高温胁迫引起的氧化损伤。

3.3　$NaHCO_3$对慢性高温胁迫下大口黑鲈肠道热休克蛋白和炎症基因表达的影响

在热应激条件下, 热休克蛋白(HSP)通过不同的机制和作用方式共同维护细胞的正常生理功能和稳定性,被认为是细胞抵抗损伤和适应环境应激能力的重要指标[36]。Hsp90是HSP家族中分布最广泛的成员, 具有维持细胞结构完整性、重新折叠异常折叠的蛋白质和提高细胞耐热性等作用[26]。在中华绒螯蟹[26]、虹鳟(*Oncorhynchus mykiss*)[37]和尼罗罗非鱼(*Oreochromis niloticus*)[38]中, 高温胁迫增加了*hsp90*的基因表达。本实验中, 与对照组相比, 饲料中添加$NaHCO_3$显著降低了大口黑鲈肠道热休克蛋白(*hsp60*、*hsp70*和*hsp90*)的基因表达, 表明$NaHCO_3$缓解了高温胁迫引起的肠道损伤[13]。研究表明, 过高的温度刺激则会影响尼罗罗非鱼肠道的免疫系统[39]。高温胁迫上调了虹鳟[37]、杂交鲟[8]和尼罗罗非鱼[39]肠道抗炎因子的表达, 引起了肠道氧化损伤。在慢性高温胁迫下, 添加$NaHCO_3$显著下调了大口黑鲈促炎症细胞因子(*il-1β*、*tnfα*和*il15*)的基因表达, 上调了抗炎细胞因子*il10*的表达, 表明

$NaHCO_3$可能具有抗炎作用。

3.4 $NaHCO_3$对慢性高温胁迫下大口黑鲈肠道组织学及紧密连接相关基因表达的影响

高温胁迫可能会通过影响鱼类消化酶的活性, 改变肠道吸收能力, 使其对食物的消化能力下降[28, 40, 41]。在本实验中, $NaHCO_3$添加后显著提高了大口黑鲈肠道淀粉酶和胰蛋白酶的活性, 可能是提高饲料效率的原因之一。肠道屏障是机体抵御外来入侵的关键。肠黏膜屏障损伤后, 外界环境中的有害病原菌及外来毒素可侵入肠黏膜, 进而对宿主的免疫功能产生不利影响[42]。肠道黏膜层表面的褶皱和微绒毛, 在营养物质的消化和吸收中发挥着关键的作用。热应激胁迫下, 虹鳟肠道绒毛高度、肌层厚度以及杯状细胞数量均显著减少[37]。与对照组相比, 慢性高温胁迫8周后, 大口黑鲈肠道受损, 部分黏膜细胞脱落。而在饲料中添加了$NaHCO_3$后则改善了肠道组织学形态, 增加了绒毛高度、肌层厚度以及杯状细胞数量。紧密连接蛋白主要由外周膜蛋白ZO-1和跨膜蛋白OCLN和claudins组成, 是肠黏膜屏障的重要组成部分, 在维持细胞通透性和屏障功能方面发挥着关键作用[43]。因此, 紧密连接蛋白的下调将对肠道结构和屏障功能产生不利影响。在虹鳟中, 热应激显著下调了肠道中*ZO-1*、*occludin*和*claudin-8*的基因表达[37]。慢性高温胁迫下, 大口黑鲈肠道*zo1*、*jam*、*occludin*、*muc2*的表达水平显著增加。结合肠道形态学结果表明饲料中添加$NaHCO_3$有效缓解大口黑鲈高温应激引起的肠道屏障损伤, 从而提高慢性高温胁迫下大口黑鲈的肠道健康。

4 结论

综上所述, 本研究探讨了饲料中添加5 g/kg的$NaHCO_3$对慢性高温应激引起的大口黑鲈肠道损伤的缓解作用。结果表明, 在慢性高温应激下, 饲料中添加$NaHCO_3$通过改善肠道屏障功能、减轻肠道氧化应激和炎症反应, 有效缓解了高温诱导的大口黑鲈肠道损伤, 从而提高了大口黑鲈的饲料利用。本研究为改善鱼类慢性高温胁迫下的肠道健康提供了理论依据。

(作者声明本文符合出版伦理要求)

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SODIUM BICARBONATE ON GROWTH PERFORMANCE, BLOOD BIOCHEMICAL PARAMERERS, AND INTESTINAL DAMAGE OF LARGEMOUTH BASS (*MICROPTERUS SALMOIDES*) UNDER CHRONIC HEAT STRESS

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Abstract: In order to investigate the effect of sodium bicarbonate ($NaHCO_3$) on intestinal damage in largemouth bass (*Micropterus salmoides*) under chronic high-temperature stress, juvenile largemouth bass (20.26±0.08) g were fed diets with or without sodium bicarbonate ($NaHCO_3$, 5 g/kg) for 8 weeks. The results showed that there was no significant difference in the specific growth rate in the $NaHCO_3$ group compared to the control group at chronic high temperatures. However, the $NaHCO_3$ group exhibited significantly improved feed efficiency, protein deposition rate, and protein efficiency, along with a marked increase in intestinal amylase and trypsin activities. The addition of $NaHCO_3$ also significantly enhanced the intestinal total antioxidant capacity (T-AOC), catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) activities. Furthermore, $NaHCO_3$ up-regulated the expression of *cat* and *gpx1a* genes and down-regulated the gene expression of *keap1*. Meanwhile, the expressions of pro-inflammatory cytokines (*il-1β*, *tnfα*, *il15*) were significantly down-regulated in the group, while the expression of anti-inflammatory cytokine *il10* was up-regulated. In addition, the expression of *hsp90*, *hsp70*, and *hsp60* genes in the intestines of largemouth bass in the $NaHCO_3$ group was significantly lower than that in the control group. Histologic analysis of the intestinal tract showed that high-temperature stress induced intestinal damage in largemouth bass, and the addition of $NaHCO_3$ significantly improved the intestinal tissue morphology and up-regulated the expression of intestinal *zo1*, *jam*, *occludin*, and *muc2*. In conclusion, the addition of 5 g/kg $NaHCO_3$ to the feed effectively alleviated chronic high-temperature stress-induced intestinal damage in largemouth bass by improving intestinal antioxidant capacity, intestinal inflammation, and intestinal barrier function. These findings may provide a new perspective to improve the intestinal health of fish under high-temperature stress.

Key words: $NaHCO_3$; Heat stress; Growth performance; Gut health; Largemouth bass