文章编号：1000-0615(2020)02-0289-11　　水产学报, 2020, 44(2): 289−299

JOURNAL OF FISHERIES OF CHINA

DOI: 10.11964/jfc.20181011469

牛磺酸对急性氨中毒的鲫和草鱼抗氧化及炎症相关基因表达影响的比较

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摘要：为了比较牛磺酸对急性氨中毒的鲫和草鱼缓释作用的差异，实验分别构建了4个处理组，组1实验鱼通过腹腔注射生理盐水，组2注射醋酸铵(鲫 7 mmol/g，草鱼 9 mmol/g)，组3注射醋酸铵和牛磺酸(100 μg/g)，组4注射牛磺酸。毒性实验持续96 h。结果显示，组2鲫肝脏中*SOD*、*CuZnSOD*和*CAT*基因mRNA表达量显著低于组1和组3；组2和组3鲫肝脏中*GPx*基因mRNA表达量显著低于组1；组1鲫大脑中*SOD*、*CuZnSOD*、*CAT*和*GPx*基因mRNA表达量最高；组2草鱼肝脏中*SOD*、*CuZnSOD*、*CAT*和*GPx*基因mRNA表达量显著高于其他组；组2和组4草鱼大脑中*SOD*和*CuZnSOD*基因mRNA表达量显著低于组1和组3；组3草鱼大脑中*CAT*和*GPx*基因mRNA表达量最高；此外，组2鲫和草鱼肝脏及大脑中*TNF*和*IL*基因mRNA表达量均显著高于其他组。研究表明，鱼类氨中毒会扰乱机体的抗氧化酶系统和免疫应答，引起氧化损伤和炎症反应；草鱼通过提高抗氧化相关基因表达以应对氨中毒；牛磺酸能够有效缓解氨中毒对鲫和草鱼造成的氧化伤害，但牛磺酸并不能降低氨中毒对鲫和草鱼造成的炎症反应。

关键词：鲫；草鱼；氨中毒；牛磺酸；氧化损伤；炎症反应

中图分类号：Q 786; S 917.4　　**文献标志码：**A

随着集约化养殖业的迅猛发展，养殖密度过高、投喂方式粗放、配合饲料营养不均衡等致使养殖水体中氨氮浓度超标成为一种常态化的环境问题[1]。氨氮在水环境中通常以离子态(NH_4^+)和非离子态(NH_3)形式存在，而非离子氨已被证实对所有的脊椎动物均具有致毒性[2]。大多数硬骨鱼对氨氮非常敏感[3]。环境中(外源性)的氨经鳃和皮肤进入鱼体后，干扰内源性氨排泄，导致谷氨酰胺在大脑中大量积累，通过激活N-甲基-D-天冬氨酸(NMDA)受体产生大量自由基(ROS)造成氧化损伤和炎症反应[4]，继而引起昏厥、抽搐，甚至死亡。在漫长的进化岁月里，鱼类逐渐分化出多种应对氨氮的生理解毒策略，归结起来可分为4种方式：①直接排氨；②减少内源性氨的产生；③尿素循环(OUC)；④谷氨酰胺(Gln)合成[5-6]。综合已有文献发现，鱼类氨中毒与氧化损伤诱发的炎症反应可能存在密切的联系。

正常生理条件下，鱼类依赖于抗氧化酶系统清除ROS：超氧化物歧化酶(SOD)催化O_2^-生成H_2O_2，而H_2O_2在过氧化氢酶(CAT)或谷胱甘肽过氧化物酶(GPx)的作用下生成无毒的H_2O和O_2[7]。氨中毒产生的ROS如果不能被鱼类抗氧化酶系统完全清除，在体内过度积累将会造成氧化损伤[8]。在不同鱼类中，抗氧化酶的活性存在很大的差异，这种差异取决于动物对胁迫因子的耐受能

收稿日期：2018-10-02　　**修回日期：**2018-11-14

资助项目：国家自然科学基金(31872541，31872222)

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力[9-10]。据此推测，鱼类应对氨中毒的抗氧化酶的活性上限因种而异[6]。此外，ROS不仅能够诱导氧化损伤，还会启动炎症反应[11]。肿瘤坏死因子(TNF)是一种促炎性细胞因子，能够启动NF-κB细胞通路，继而激活一系列炎症因子，如一氧化氮合酶(iNOS)和白介素(IL)[12]。总而言之，鱼类氨中毒将诱发氧化损伤和炎症反应，但其分子机制并不是很清楚。

牛磺酸(2-aminoethanesulfonic acid)是一种潜在的促炎性反应调节剂，发挥渗透剂、抗氧化剂和神经递质抑制剂作用，能够缓解由谷氨酰胺积累引起的脑水肿，降低炎症反应对机体造成的损伤，调节钙稳态，清除自由基，保护组织免受氧化损伤[13-14]。在哺乳动物研究中发现，高血氨症的典型症状就是牛磺酸缺乏[15]。在鱼类研究中，Takagi等[16]发现，饲料中牛磺酸缺乏会造成五条鲷(*Seriola quinqueradiata*)溶血性贫血，导致血浆牛磺酸含量和红细胞渗透压调节能力下降。Salze等[17]报道，北美鲳鲹(*Trachinotus carolinus*)摄食牛磺酸缺乏的饲料16 d后，肝脏线粒体蛋白含量和抗氧化酶活性显著下降。高密度养殖系统极易形成氨氮胁迫，使得鱼类高血氨症频发，已成为制约鱼类健康养殖的主要因素之一[1]。然而，牛磺酸对不同鱼类高血氨症的缓释作用机制，迄今尚不十分清楚。

本实验旨在验证两个可能的假设：①基于抗氧化基因(*SOD*、*CuZnSOD*、*CAT*和*GPx*)和炎症基因(*TNF*和*IL*)的mRNA表达水平，氨中毒对鲫(*Carassius auratus*)和草鱼(*Ctenopharyngodon idella*)造成的氧化损伤和炎症反应可能存在差异；②牛磺酸对鱼类高血氨症具有缓释作用。

1 材料与方法

1.1 实验动物及养殖管理

实验用鱼均购自浙江宁波路林水产市场，暂养14 d后，分别挑选360尾大小均匀、体格健壮的平均体质量为(42.13±2.05) g的鲫和平均体质量为(40.30±4.33) g的草鱼，并随机分配到12个300 L塑料养殖桶中(3个重复)，每缸30尾。整个实验过程中，养殖用水为除氯自来水，日换水量为总水体的1/3，气石持续充氧，水温维持在27~29 °C，溶解氧(7.81±0.13) mg/L，pH 7.2~7.4，亚硝酸盐含量<0.5 mg/L，保持自然光照。

1.2 实验设计及样品采集

鲫和草鱼各分为4个处理组：组1(对照组)腹腔注射0.2 mL 0.9% 生理盐水；组2腹腔注射0.2 mL半致死浓度(LC_{50})的醋酸铵(鲫 7 mmol/g，草鱼 9 mmol/g)[18-19]；组3在注射0.2 mL LC_{50}醋酸铵前10 min腹腔注射0.2 mL牛磺酸(100 μg/g鱼体质量[4])；组4腹腔注射0.2 mL牛磺酸(100 μg/g鱼体质量)。急性毒性实验持续96 h。

实验结束后，采用MS-222麻醉，每缸随机挑选3尾存活的实验鱼(每缸均>15尾)，冰上解剖获得大脑和肝脏样品，经液氮快速冷冻后，−80 °C保存备用，用于分析相关基因的mRNA表达。

1.3 总RNA提取和cDNA合成

取0.5 g冷冻的大脑或肝脏组织于RNAiso Plus [宝生物工程(大连)有限公司]中匀浆，向匀浆液中加入约1/5体积的氯仿，4 °C离心15 min (12 000 r/min)，取上清液，加入等体积的异丙醇溶液，4 °C离心10 min(12 000 r/min)，弃上清液，加入75 %乙醇洗涤2遍，干燥后溶于适量DEPC水中。通过OD_{260}/OD_{280}比值确定RNA浓度和纯度，1%琼脂糖凝胶电泳分析RNA完整度。取1 μL总RNA，按照TransScript All-in-one First-Strand cDNA Synthesis SuperMix for qPCR (One-Step gDNA Removal)试剂盒(北京全式金生物技术有限公司)说明书完成第一链cDNA的合成。

1.4 mRNA组织表达量

基于前期工作获得鲫和草鱼转录组数据库中的抗氧化基因(*SOD*、*CuZnSOD*、*CAT*和*GPx*)和炎症基因(*TNF*和*IL*)序列片段，以β-肌动蛋白基因(*β-actin*)为内参基因，采用Primer Premier 5.0软件设计引物(表1)。实时荧光定量PCR (qRT-PCR)反应体系(20 μL)：10 μL TransStart Tip Green qPCR SuperMix (2×)、0.4 μL正向引物、0.4 μL反向引物、0.4 μL cDNA模板、8.8 μL无菌水。反应条件：94 °C 2 min；94 °C 30 s，55 °C 30 s，72 °C 30 s，40个循环；72 °C 10 min，每个反应3次重复。采用相对定量$2^{-\Delta\Delta C_T}$法[20]计算目的基因的相对表达量。

1.5 统计分析

实验数据采用单因素方差分析(One-Way ANOVA)进行统计学处理，如果各处理组间差异

表 1　鲫(C)和草鱼(G)实时荧光定量PCR引物序列

Tab. 1　Primers used for Real-time PCR analysis from *C. auratus* (C) and *C. idella* (G)

目的基因 aim genes	正向引物 (5′-3′) forward primers (5′-3′)	反向引物 (3′-5′) reverse primers (5′-3′)	大小/bp size
C-*SOD*	CACGGTGGACCAACTGAT	CCTCCCAATGACGGAGTA	135
C-*CuZnSOD*	ACTGGCAACGCTGGAGGT	GTGGGCTAAGTGCTAATGGA	133
C-*CAT*	GCAAAGCCAAAGTGTTCG	TGAAGGACGGAAACAGAA	210
C-*GPX*	CGACTCCGTGTCCTTGAT	GTTTATTTCGCCCTTGAT	192
C-*IL*	AACGAATCCCAGACTTCACACT	GGCATAGAAAAAGACAACAAA	198
C-*TNF*	TGAAATTAGCGGGAAGGGAG	GCCATAGGAATCAGAGTAGCGG	157
C-*β-actin*	ATGCGGAAACTGGAAAGG	AGGGCAGAGTGGTAGACG	115
G-*SOD*	GTCCGCACTTCAACCCTTACA	GACTTTCCTCATTGCCTCCCT	222
G-*CuZnSOD*	TCCGCACTTCAACCCTTACA	ACTTTCCTCATTGCCTCCCT	220
G-*CAT*	TTGCGTCCTGAATCGTTG	ATGGGAAGTTGCCGTTGG	265
G-*GPX*	GGGGCTGGTTATTCTGGG	AGGCGATGTCATTCCTGTTC	278
G-*IL*	CAAAACTTCACTCCCGCTTACA	GTTCATCGTTTGTGCGGTTC	110
G-*TNF*	AGACGACGCCACTACTCCTG	CCTCTCCACAGGTTTCCATTC	135
G-*β-actin*	GCCGTGACCTGACTGACT	CAAGACTCCATACCCAAGAA	271

显著(P<0.05)，则进行Tukey多重比较。所有分析均采用SPSS 18.0.0软件 (Chicago，USA)在Windows操作系统中进行。

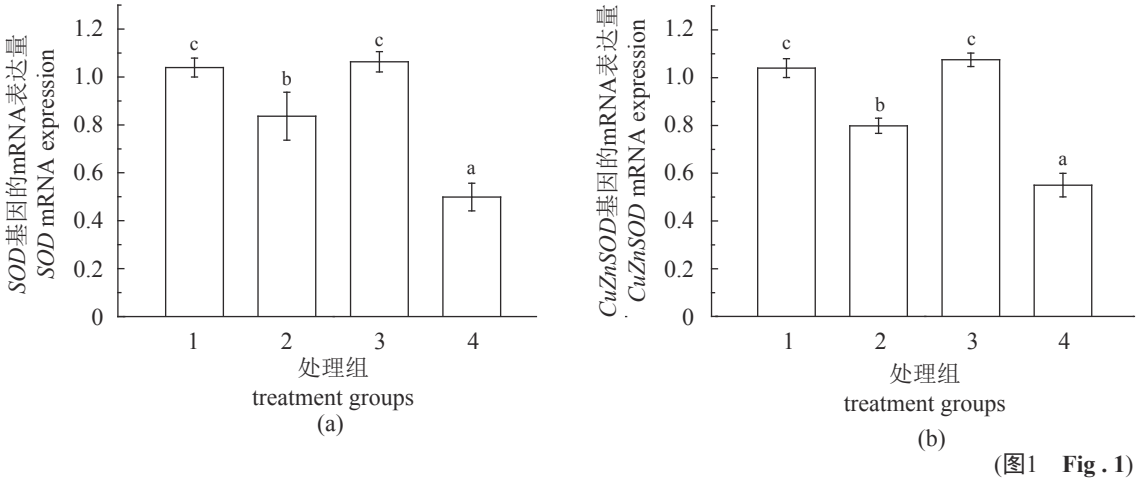
2　结果

组2鲫肝脏中*SOD*、*CuZnSOD*和*CAT*基因的mRNA表达量显著低于组1和组3(P<0.05)(图1-a，b，c)；组2和组3 *GPx*基因的mRNA表达量无显著性差异(P>0.05)，均显著低于组1，高于组4(P<0.05)(图1-d)。

组1鲫大脑中*SOD*、*CuZnSOD*、*CAT*和*GPx*基因的mRNA表达量最高(P<0.05)(图2)，而其他处理组间无显著性差异(P>0.05)。

组2草鱼肝脏中*SOD*、*CuZnSOD*、*CAT*和*GPx*基因的mRNA表达量最高，组4最低(P<0.05)(图3)。

组1草鱼大脑中*SOD*和*CuZnSOD*基因的mRNA表达量显著最高(P<0.05)(图4-a，b)，其次是组3，组2和组4最低；组3草鱼大脑中*CAT*和*GPx*基因的mRNA表达量显著高于其他处理组(P<0.05)(图4-c，d)。



(图1　**Fig . 1**)

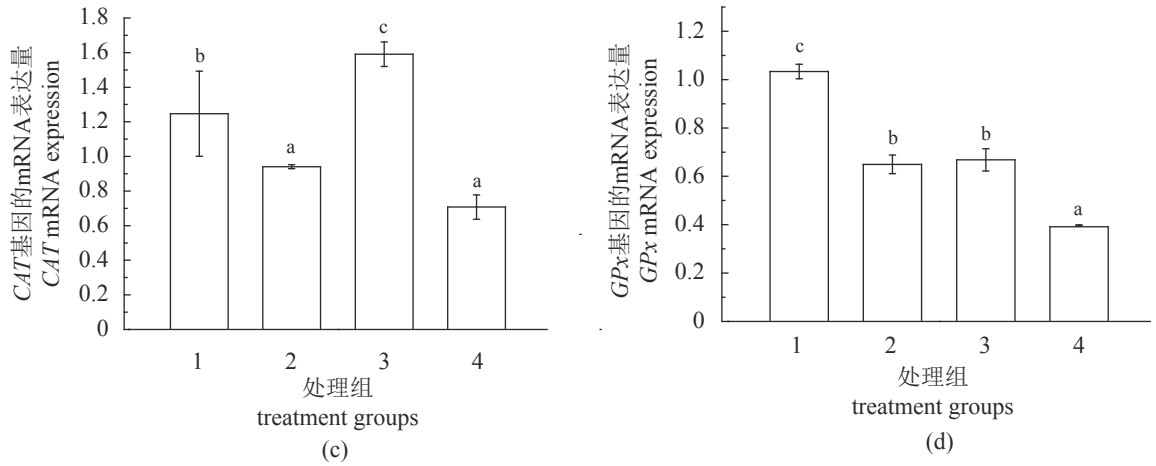


图 1 醋酸铵和牛磺酸对鲫肝脏中*SOD*、*CuZnSOD*、*CAT*和*GPx*基因mRNA表达量的影响

1. 生理盐水组，2. 醋酸铵组，3. 醋酸铵和牛磺酸组，4. 牛磺酸组；不同字母表示差异显著(P<0.05)；下同

Fig. 1 Effects of CH_3COONH_3 and taurine on mRNA expression of *SOD*, *CuZnSOD*, *CAT* and *GPx* in liver of *C. auratus*

1. NaCl group, 2. ammonium acetate group, 3. ammonium acetate and taurine group, 4. taurine group; different lowercase letters indicate significant difference (P<0.05); the same below

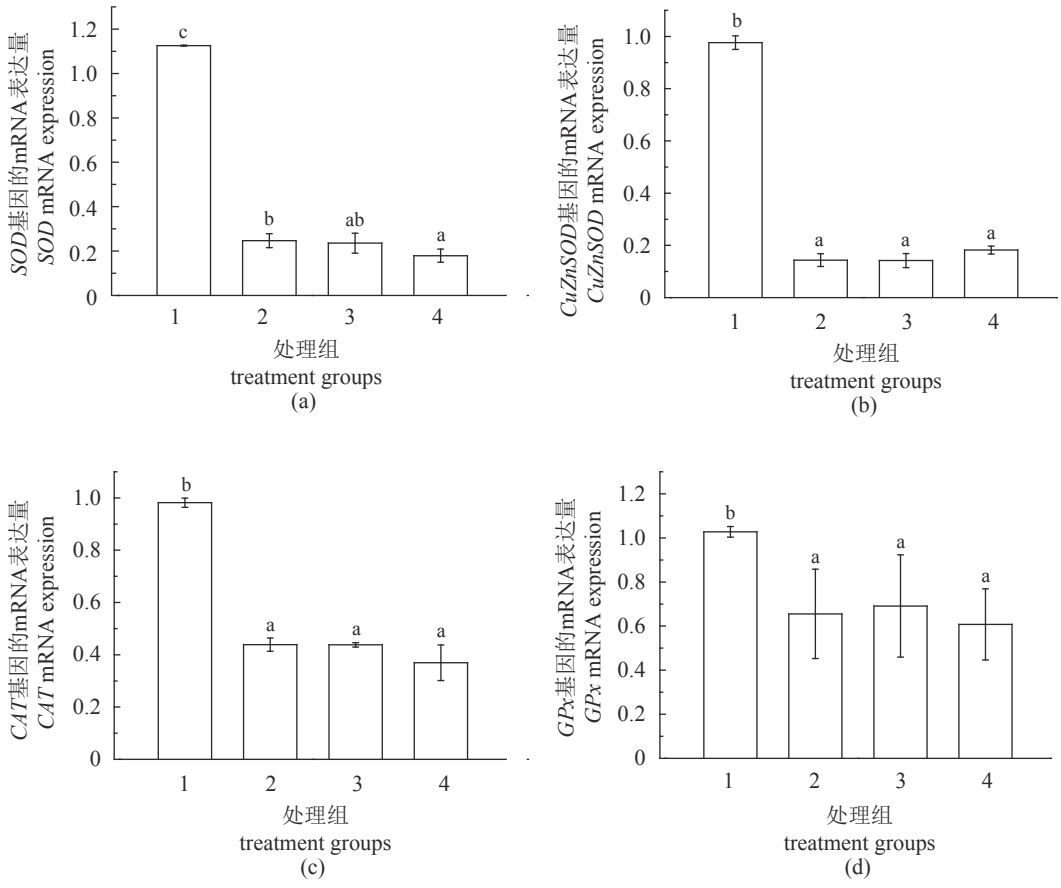


图 2 醋酸铵和牛磺酸对鲫大脑中*SOD*、*CuZnSOD*、*CAT*和*GPx*基因mRNA表达量的影响

Fig. 2 Effects of CH_3COONH_3 and taurine on mRNA expression of *SOD*, *CuZnSOD*, *CAT* and *GPx* in brain of *C. auratus*

组2鲫肝脏和大脑中*TNF*和*IL*基因的mRNA表达量显著高于其他组(P<0.05)(图5，图6)，其次是组1、组3和4最低。

组2草鱼肝脏中*TNF*和*IL*基因的mRNA表达量显著高于其他组(P<0.05)(图7)；组2草鱼大脑中*TNF*和*IL*基因的mRNA表达量最高，其次是组1，最低是组3和组4(P<0.05)(图8)。

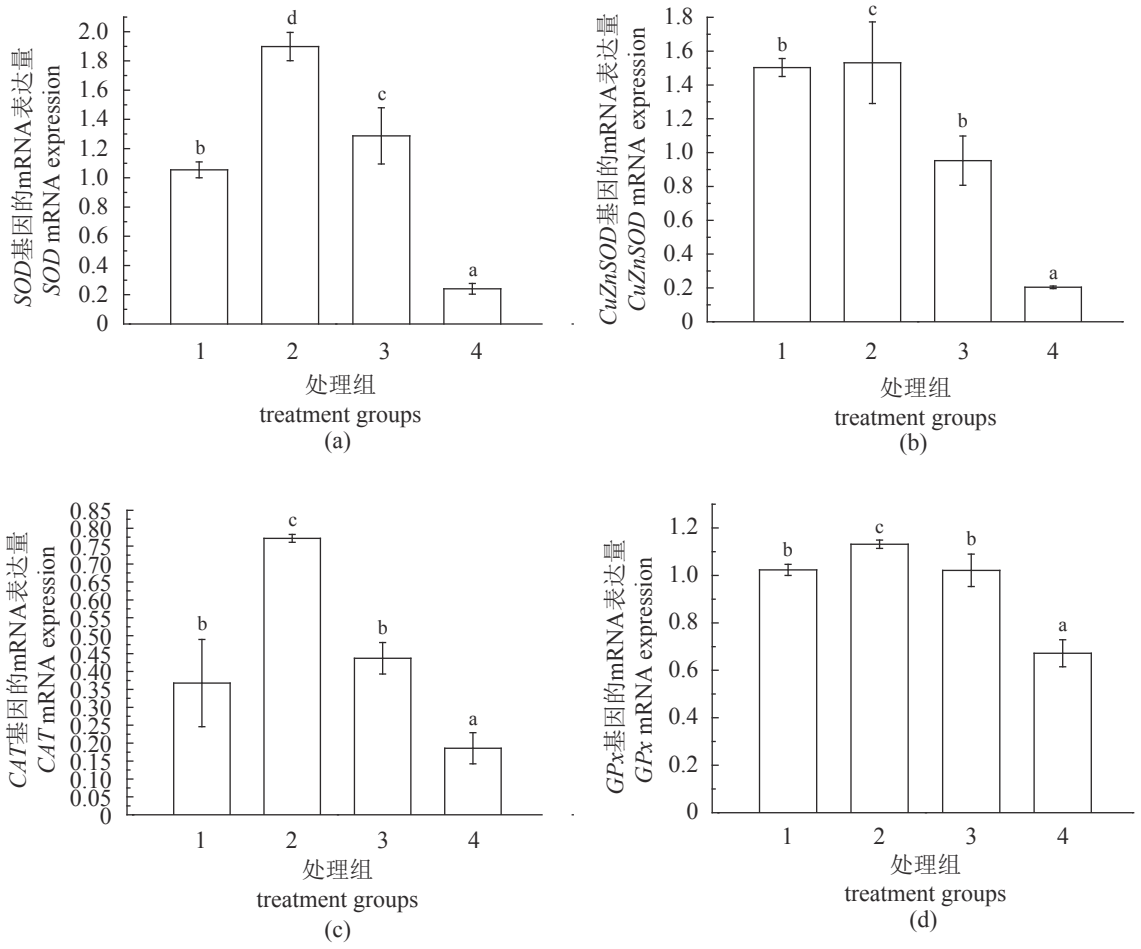
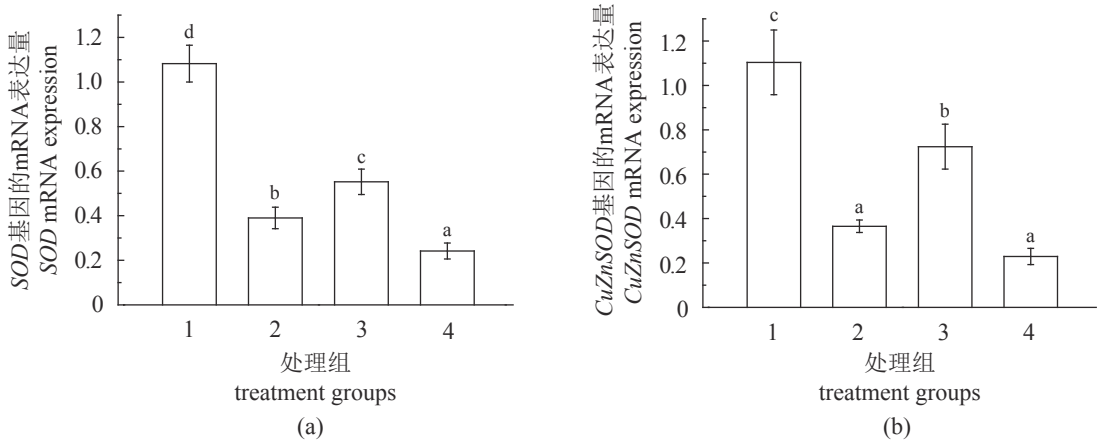


图 3　醋酸铵和牛磺酸对草鱼肝脏中*SOD*、*CuZnSOD*、*CAT*和*GPx*基因mRNA表达量的影响

Fig. 3　Effects of CH_3COONH_3 and taurine on mRNA expression of *SOD*, *CuZnSOD*, *CAT* and *GPx* in liver of *C. idella*



(图4　**Fig . 4**)

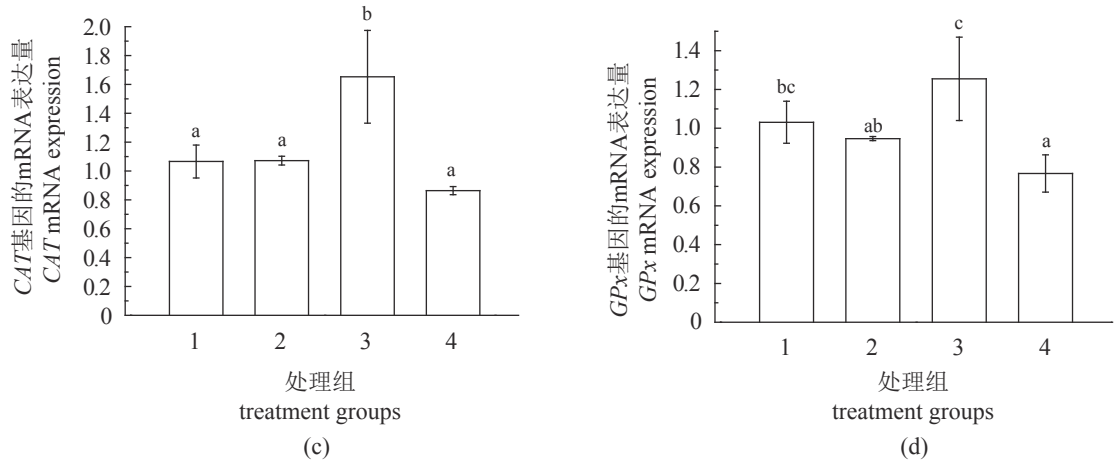


图 4 醋酸铵和牛磺酸对草鱼大脑中*SOD*、*CuZnSOD*、*CAT*和*GPx*基因mRNA表达量的影响

Fig. 4 Effects of CH_3COONH_3 and taurine on mRNA expression of *SOD*, *CuZnSOD*, *CAT* and *GPx* in brain of *C. idella*

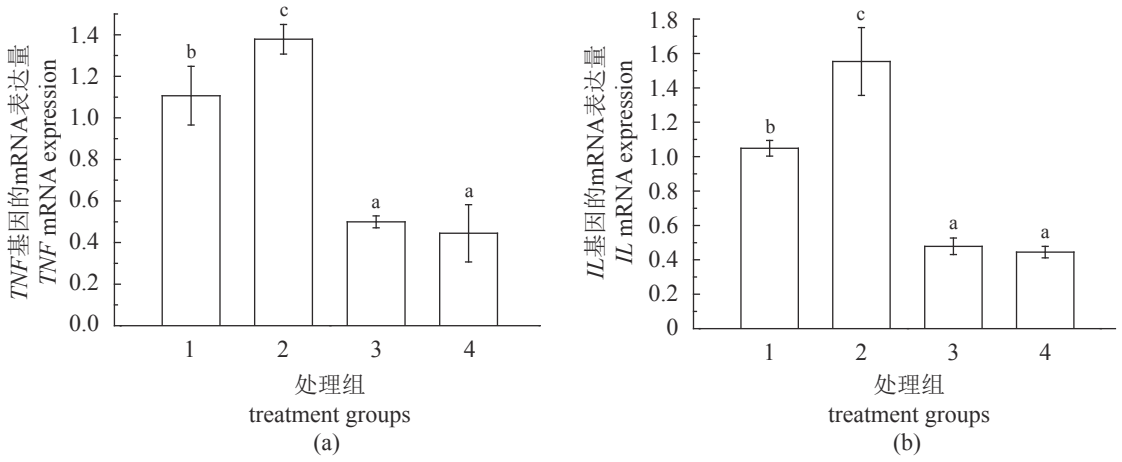


图 5 醋酸铵和牛磺酸对鲫肝脏中*TNF*和*IL*基因mRNA表达量的影响

Fig. 5 Effects of CH_3COONH_3 and taurine on mRNA expression of *TNF* and *IL* in liver of *C. auratus*

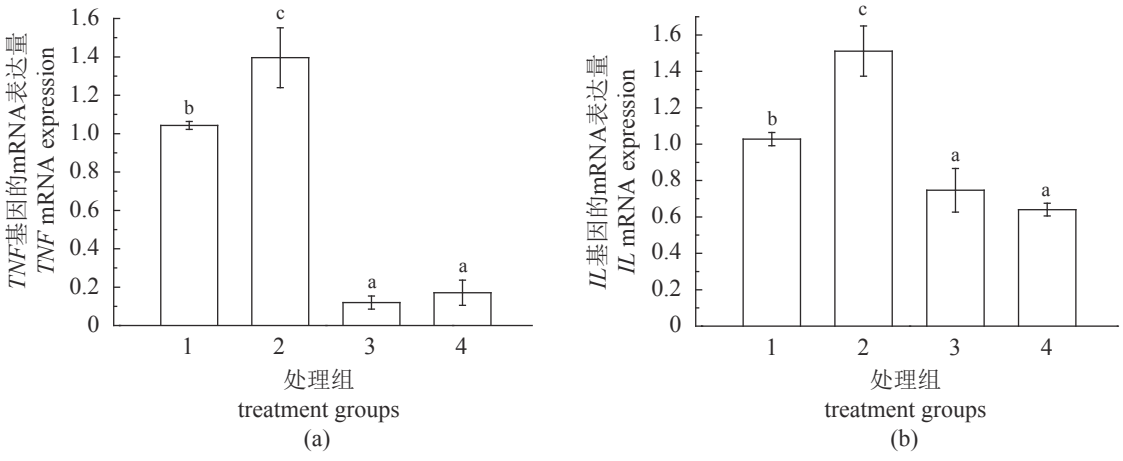


图 6 醋酸铵和牛磺酸对鲫大脑中*TNF*和*IL*基因mRNA表达量的影响

Fig. 6 Effects of CH_3COONH_3 and taurine on mRNA expression of *TNF* and *IL* in brain of *C. auratus*

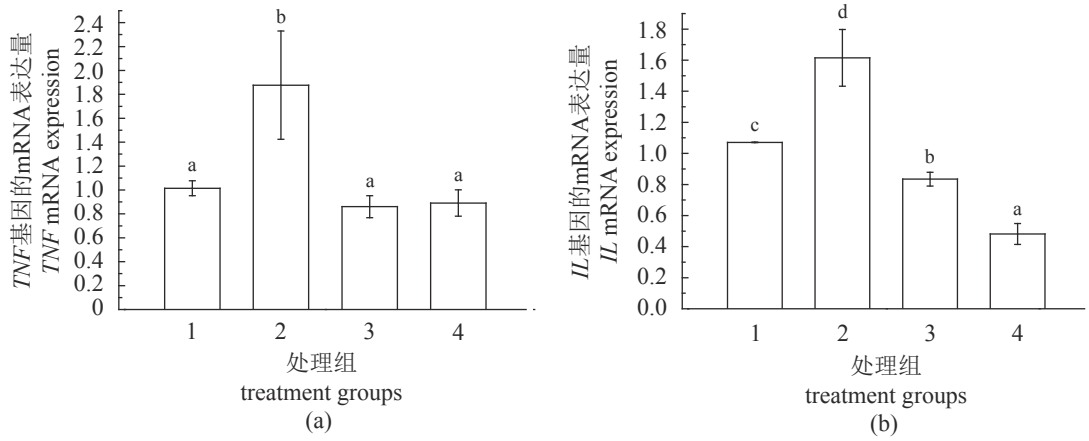


图 7　醋酸铵和牛磺酸对草鱼肝脏中*TNF*和*IL*基因mRNA表达量的影响

Fig. 7　Effects of CH_3COONH_3 and taurine on mRNA expression of *TNF* and *IL* in liver of *C. idella*

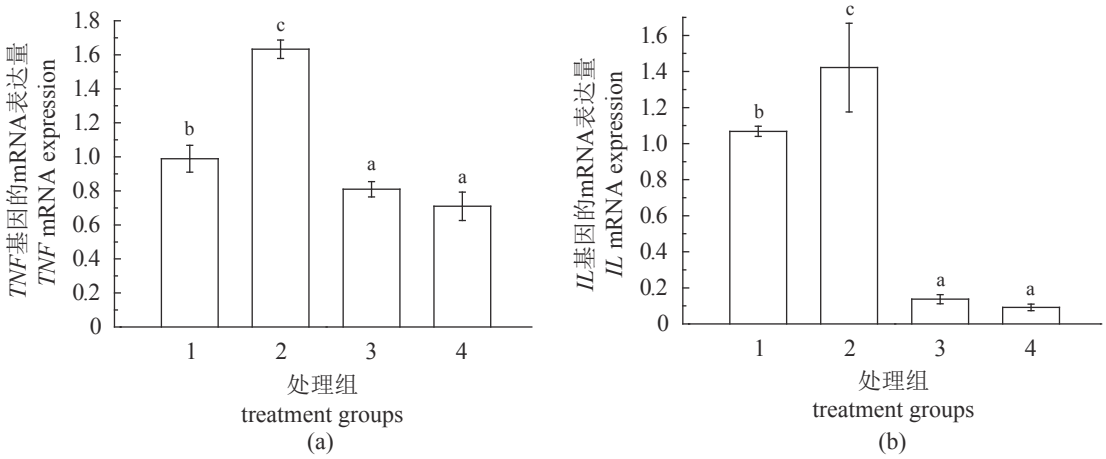


图 8　醋酸铵和牛磺酸对草鱼大脑中*TNF*和*IL*基因mRNA表达量的影响

Fig. 8　Effects of CH_3COONH_3 and taurine on mRNA expression of *TNF* and *IL* in brain of *C. idella*

3　讨论

大量研究表明，氨氮能够在动物体的不同组织中积累从而导致中毒症状，例如金头鲷(*Sparus aurata*)、大菱鲆(*Scophthalmus maximus*)[21]和许氏平鲉(*Sebastes schlegelii*)[22]的血液和肝脏中，细鳞非洲肺鱼(*Protopterus dolloi*)[23]和暗纹多纪鲀(*Takifugu obscurus*)[24]的肌肉和肝脏中，草鱼[19]、鲫[18]和黄颡鱼(*Pelteobagrus fulvidraco*)[25]的大脑和肝脏中。从已有研究来看，肝脏作为机体生理解毒的主要器官，是开展鱼类毒理研究的关键组织。鱼类氨中毒会过度激活NMDA生成大量ROS，继而导致蛋白结构破坏和细胞膜过氧化的发生[26-27]。通常情况下，动物依赖自身抗氧化酶体系，如超氧化物歧化酶、过氧化物酶和谷胱甘肽过氧化物酶等，能够耐受一定程度的ROS，但抗氧化酶的活性往往受到体内和体外环境的共同影响，从而表现出不同程度的差异[8, 28]。本研究发现，组2鲫肝脏中*SOD*、*CuZnSOD*、*CAT*和*GPx*基因的mRNA表达量显著低于组1，可能是由于氨中毒抑制了机体抗氧化酶的活性。类似的发现在其他鱼类中也屡有报道，如尼罗罗非鱼(*Oreochromis niloticus*)[28]、许氏平鲉[22]、鳙(*Hypophthalmichthys nobilis*)[29]、鲫[18]和黄颡鱼[25]。然而，在本研究中，氨中毒却上调了草鱼肝脏中抗氧化酶相关基因的mRNA表达量，这可能是草鱼有别于其他鱼类耐受高血氨水平的一种生理适应性调节，以此减少氨氮毒性对机体造成的氧化损伤。Jin等[30]发现，草鱼暴露在高氨氮环境中，肝脏中SOD活性响应在2 h内发生，并在24 h内迅速达到峰值。综合以上研究结果推测，草鱼有别于其他鱼类而具有较强的氨氮适应力，

可能与体内抗氧化酶具有更强的氨氮耐受性有关。此外，在虹鳟(*Oncorhynchus mykiss*)，鲤(*Cyprinus carpio*)，金鱼[31]和舌齿鲈(*Dicentrarchus labrax*)[32]等鱼类的研究中，发现其肝脏中SOD活性并未受到氨氮暴露的影响。综上，由于氨氮耐受力的差异，不同鱼类暴露到氨氮环境中，其抗氧化酶体系的生理响应并不完全一致。

牛磺酸参与机体多种重要的生理活动，如组织修复、抗氧化、免疫调节、重金属解毒、渗透压调节和神经调节等[13-14]。在哺乳动物研究中发现，牛磺酸缺乏是诱发高血氨症诸多因素中的主要因素之一。Chepkova等[15]证实牛磺酸缓解小鼠(*Mus musculus*)高血氨症的作用机制，是通过提高线粒体活性，减少氨氮和谷氨酰胺的积累。本研究发现，同时注射了醋酸铵和牛磺酸的组3与对照组鲫肝脏中*SOD*和*CuZnSOD*及草鱼肝脏中*CAT*和*GPx*基因mRNA表达量无显著性差异，结果提示，外源牛磺酸能够对鱼类氨中毒发挥有效的缓释作用。Smart[33]提出，牛磺酸所具有的渗透压调节功能，能够缓解大脑水肿，很可能是其发挥氨氮毒性缓释作用的关键所在。Li等[34]在其研究中进一步证实，腹腔注射LC_{50}醋酸铵的黄颡鱼，其死亡率与所食用饲料中牛磺酸的含量呈负相关。

与肝脏中抗氧化相关基因表达结果相似，氨中毒诱导了鲫和草鱼大脑中*SOD*和*CuZnSOD*基因表达下调。氨氮在肝脏中可以被转化为尿素，但肝脏的解毒功效存在耐受上限，未被解毒的氨氮将突破血脑屏障，造成脑组织损伤[35]。综上所述，氨氮引起的脑组织氧化损伤是诱发哺乳动物高血氨症的主要原因之一，其典型症状为脑水肿、氧化损伤及免疫抑制[36-37]。Sochor等[38]发现牛磺酸能够在动物体不同组织中发挥抗氧化作用，可有效阻止生物膜过氧化的发生。牛磺酸在中枢神经系统中含量较高，通过调节渗透压平衡能够缓解大脑星形细胞肿胀[39]。本研究发现，鲫大脑中抗氧化酶相关基因的表达并没有受到牛磺酸的影响，但牛磺酸却显著上调了草鱼大脑中抗氧化相关基因的表达，结果提示，相比鲫，草鱼的脑组织对牛磺酸更加敏感。

氨中毒还会诱导机体的炎症反应[11]。本研究发现，氨中毒的鲫和草鱼肝脏和大脑中*TNF*和*IL*基因的表达量显著上调。Cheng等[24]在暗纹多纪鲀中的研究中也有类似的发现：氨氮胁迫提高了炎症反应相关基因(*TNF-a*、*IL*-6和*IL*-12)的转录水平。TNF是一种多效促炎性因子，参与炎症反应、细胞凋亡、细胞增生及免疫过激等生理过程，TNF还能够激活NF-κB细胞通路[40]。已有研究表明，牛磺酸参与了机体TNF等炎症因子释放的调节[14]，在一项针对小鼠的研究中证实，牛磺酸能够有效地缓解高血氨症引起的炎症反应[15]。然而，在本研究中却发现，氨中毒的鲫和草鱼肝脏和大脑中*TNF*和*IL*基因的表达量并未受到牛磺酸的影响。

综上，鱼类氨中毒会扰乱机体的抗氧化酶系统和免疫应答，引起氧化损伤和炎症反应；草鱼通过提高抗氧化相关基因表达以应对氨中毒；牛磺酸能够有效缓解氨中毒对鲫和草鱼造成的氧化伤害；但牛磺酸并不能降低氨中毒对鲫和草鱼造成的炎症。

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Comparison effects of taurine on acute ammonia toxicity in genes involved in antioxidant and inflammation of *Carassius auratus* and *Ctenopharyngodon idella*

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Abstract: The four experimental groups were set up to test the response of *Carassius auratus* and *Ctenopharyngodon idella* to ammonia toxicity and taurine within 96 h. Group 1 was injected with NaCl, group 2 was injected with ammonium acetate (*C. auratus* 7 mmol/g; *C. idella* 9 mmol/g), group 3 was injected with ammonium acetate and taurine (100 μg/g), and group 4 was injected with taurine. *C. auratus* in group 2 had lower mRNA expression of *SOD*, *CuZnSOD* and *CAT* in liver than those of tested fish in groups 1 and 3; tested fish in groups 2 and 3 had lower mRNA expression of *GPx* in liver than that in group 1; the highest mRNA expression of *SOD*, *CuZnSOD*, *CAT* and *GPx* in brain were found in group 1; *C. idella* in groups 2 had higher mRNA expression of *SOD*, *CuZnSOD*, *CAT* and *GPx* in liver; fish in groups 2 and 4 had lower mRNA expression of *SOD* and *CuZnSOD* in brain than those in groups 1 and 3; fish in group 3 had the highest mRNA expression of *CAT* and *GPx* in brain. *C. auratus* and *C. idella* in group 2 had the highest mRNA expression of *TNF* and *IL* in the brain and liver. This study indicates that defensive strategies are more effectively on *C. idella* in dealing with the ammonia challenge compared with *C. auratus*; the taurine could more effectively mitigate the adverse effect of oxidative stress on *C. auratus* and *C. idella*, but the inflammatory response in hyperammonemia *C. auratus* and *C. idella* was not alleviated by taurine.

Key words: *Carassius auratus*; *Ctenopharyngodon idella*; ammonia toxicity; taurine; oxidative stress; inflammation

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Funding projects: National Natural Science Foundation of China (31872541, 31872222)