



急性氨氮胁迫对黄颡鱼组织抗氧化酶活性及HSP70和HSP90基因mRNA表达水平的影响

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摘要: 为了探讨急性氨氮胁迫对黄颡鱼组织中抗氧化酶活性及HSP70和HSP90基因mRNA表达水平的影响, 实验随机挑选了360尾黄颡鱼[初体质量(17.25 ± 0.05) g], 分别暴露于含有0(对照)、5.70(低浓度组)、28.50(中浓度组)和57.00(高浓度组) mg/L总氨氮浓度的水体中, 进行96 h的急性胁迫实验。实验开始后, 分别于0、12、24、48和96 h取样。结果显示, 氨氮胁迫发生后, 低、中浓度组实验鱼肝脏中超氧化物歧化酶(SOD)活性呈先升高后降低趋势, 而高浓度组则持续降低; 低、中、高浓度组实验鱼肝脏中丙二醛(MDA)含量在胁迫开始后显著升高; 3 h时, 高浓度组实验鱼肝脏中SOD活性达到最低, 而MDA含量最高; 24 h后, 高浓度组实验鱼肝脏中过氧化氢酶(CAT)活性显著升高; 低、中、高浓度组实验鱼肝脏中HSP70基因的mRNA表达量呈先降低后升高趋势, 而鳃中HSP70基因表达量持续升高, 但脑中HSP70基因在0 h后显著降低; 氨氮胁迫3 h时, 低、中、高浓度组实验鱼肝脏和脑中HSP70基因表达量显著低于对照组, 而在鳃中正好相反; 相比HSP70基因, 高氨氮浓度组实验鱼肝脏和鳃中HSP90基因的mRNA表达量在24 h时达到最高。研究表明, 不同浓度的氨氮胁迫会对黄颡鱼抗氧化酶活性造成不同程度的抑制, 原因与丙二醛的积累量有关; 相比HSP90基因, 黄颡鱼HSP70基因的表达量在氨氮胁迫发生后迅速上调, 这种生理调控机制提示HSP70在应对急性氨氮胁迫时发挥着更重要的作用。

关键词: 黄颡鱼; 氨氮; 抗氧化酶; HSP70; HSP90

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随着集约化水产养殖业的迅猛发展, 养殖水体中氨氮超标已成为一种常态化的环境问题, 绝大多数鱼类对非离子态氨(NH₃)非常敏感, 高浓度的NH₃在鱼体内大量蓄积会引起呼吸过速、过度兴奋、抽搐甚至死亡^[1-4]。在哺乳类高血氨症的研究中发现, NH₃在大脑中过度积累, 会造成星状胶质细胞肿胀, 使得N-甲基-D-天冬氨酸(NMDA)受体和Ca²⁺依赖一氧化氮合成酶途径过度激活, 继而产生大量活性自由基(ROS),

造成功能细胞氧化损伤^[5]。因此, 探究鱼类氨中毒的致病机制显得十分重要, 尤其是氨中毒^[6]与氧化损伤之间的内在联系。

先前的研究表明, 环境胁迫(如热应激、重金属、氨氮及病害等)会促进动物体内热休克蛋白(HSPs)的合成, HSPs通过阻止蛋白折叠、降解变性蛋白等途径, 有效地降低应激对机体造成的损伤^[7-10]。此外, HSPs还参与机体抗氧化酶活性的调控, 通过抑制自由基生成关键酶活性

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减少ROS的产生^[11]。HSPs按其表观分子量可划分为多种类型,如HSP47、HSP60、HSP70和HSP90等。其中,HSP70在正常细胞中不表达或表达量很少,但在应激条件下会迅速增加^[12];而HSP90通过调节一大类多肽底物的折叠,也可以在应激条件下增加热降解蛋白质的再活化比例^[13]。研究表明,HSP70和HSP90与清除变性蛋白、维持胞内蛋白质稳态、防止或减少细胞受损、促进应激细胞恢复等多种生理功能有关^[14]。Cara等^[15]发现,金头鲷(*Sparus aurata*)和虹鳟(*Oncorhynchus mykiss*)在饥饿胁迫发生后,鱼体内HSP70基因的mRNA表达量显著上调;刘波等^[16]报道,尼罗罗非鱼(*Oreochromis niloticus*)暴露于低温水环境(9℃)中12 h后,肝脏中HSP70基因的mRNA表达量显著上调;周彦静^[17]发现,虹鳟的鳃、中肾、脑、心脏和肌肉中HSP90基因的mRNA表达量随着环境温度的升高呈逐步上调的趋势。相比而言,鲜有研究报道氨氮胁迫下鱼类组织中HSPs基因表达量的变化情况,鱼类HSPs基因在氨中毒机制中的作用迄今尚不清楚。

黄颡鱼(*Pelteobagrus fulvidraco*)隶属鲇形目(Siluriformes)、鲇科(Bagridae),其肉质鲜美,含有人体所需的多种必需氨基酸,深受广大消费者的喜爱。据2018年《中国渔业统计年鉴》发布的权威数据,截止2017年底,全国黄颡鱼产量达到48万t,年增幅超过10.5%,已成为我国重要的淡水养殖种类之一^[18]。由于其具有较强的氨氮耐受力,从而成为本研究绝佳的实验对象。本实验通过分析急性氨氮胁迫对黄颡鱼肝脏中抗氧化酶的活性,以及对肝脏、鳃和脑中HSP70和HSP90基因mRNA表达量的影响,为鱼类氨中毒致死机制的深入研究提供理论依据。

1 材料与方法

1.1 养殖管理及取样

黄颡鱼购自浙江湖州,暂养7 d后,挑选360尾大小均匀的实验鱼[(17.25 ± 0.05) g],随机分配到12个300 L塑料养殖桶中,每桶30尾鱼。共设置4个氨氮处理组:0(对照)、5.70(安全浓度)、28.50(半致死浓度)和57.00 mg/L总氨氮(T-AN),胁迫周期为96 h,期间停止投喂实验饲料。氨氮浓度通过预配NH₄Cl(10 g/L)母液每隔7 h进行调整,总氨氮浓度测定采用纳氏试剂法^[19]。养殖过程中,养殖用水为除氯自来水,日换水量为总

体积的1/3,水温24~26℃,溶解氧(7.81 ± 0.13) mg/L, pH 6.4~6.6,亚硝酸盐<0.5 mg/L,保持自然光照。

实验开始后,分别于0、12、24、48和96 h取样,每次每桶随机挑选3尾实验鱼,MS-222麻醉后解剖获取肝脏、鳃和脑组织,液氮速冻后-80℃保存,用于相关基因的mRNA表达量分析,剩余肝脏-20℃保存,用于酶活性的检测。

1.2 抗氧化酶活性及脂质过氧化分析

称重肝脏样品0.1 g,在预冷的磷酸缓冲液(50 mmol/L, pH 7.4)中匀浆,匀浆液于4℃环境中2 000×g离心15 min,分离上清液备测。超氧化物歧化酶(SOD)活性测定参考Beauchamp等^[20]报道的方法;过氧化物酶(CAT)活性测定参考Aebi^[21]报道的方法;丙二醛(MDA)含量通过硫代巴比妥酸反应测定,参考Buege等^[22]报道的方法。所有测试均采用南京建成生物工程研究所的商业试剂盒分析,操作步骤严格按照说明书进行。

1.3 基因mRNA表达量分析

总RNA的提取采用RNA iso Plus试剂(TaKaRa, 大连),利用Prime Script™ PT reagent Kit with gDNA Eraser(TaKaRa, 大连)反转录成cDNA用于实时荧光定量PCR分析。利用Primer Premier 5.0软件设计引物(表1)。实时荧光定量PCR(SYBR Premix Ex Taq II, TaKaRa, 大连)扩增程序为95℃预变性5 min,40个循环包括95℃ 20 s,57℃ 25 s,72℃ 25 s。采用2^{-ΔΔC_t}法分析基因相对表达量^[23]。

1.4 数据分析

实验数据采用双因素方差分析(Two-Way ANOVA),如果各处理组间差异显著($P < 0.05$),则进行Tukey氏多重比较。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

2.1 黄颡鱼肝脏中SOD、CAT及MDA的变化

胁迫过程中,对照组实验鱼肝脏中的SOD、CAT活性及MDA含量均未发生显著性变化($P > 0.05$);随胁迫持续,低、中浓度组实验鱼肝脏中的SOD活性呈先升高后降低趋势,最高值分别出现在6和3 h;高浓度组随着胁迫的发生呈持续降低趋势($P < 0.05$);高浓度组实验鱼肝脏中的

表1 本实验用到的引物序列

Tab. 1 Sequence of primers used in this study

目的基因 aim gene	正向引物 (5'-3') forward primer (5'-3')	反向引物 (3'-5') reverse primer (3'-5')	大小/bp size
HSP70	GGCTTGCCCTTTGGGAGTC	GATTGGTCGGCGATTGA	253
HSP90	TCTCAAGTGCCCAAGTTACAT	CGTCCAGTGGTGCTTTAGGT	241
β -actin	TTCGCTGGAGATGATGCT	CGTGCTCAATGGGGTACT	136

CAT活性在24 h时达到最高($P<0.05$), 而其他实验组在胁迫过程中无显著性差异($P>0.05$); 低、中、高浓度组实验鱼肝脏中的MDA含量在整个胁迫过程中均呈显著升高趋势($P<0.05$)。氨氮胁迫0 h时, 低、中、高浓度组实验鱼肝脏中的SOD、CAT

活性及MDA含量在各处理组间无显著性差异($P>0.05$); 氨氮胁迫24 h时, 中浓度组实验鱼肝脏中的SOD活性显著高于其他组($P<0.05$); 氨氮胁迫3 h时, 中浓度组实验鱼肝脏中的MDA含量最高($P<0.05$)(表2)。

表2 不同氨氮浓度暴露96 h对黄颡鱼肝脏中抗氧化酶活性及脂质过氧化程度的影响

Tab. 2 Liver antioxidant enzyme activities and lipid peroxidation levels at different time in *P. fulvidraco* during 96-hours exposure to different concentration of environmental ammonia

组别 groups		胁迫时间/h stress time						
		0	3	6	12	24	48	96
超氧化物歧化酶(U/mg prot) superoxide dismutase	对照组 control	295.33±7.89	297.12±15.44 ^B	291.25±9.18 ^B	296.44±13.28 ^B	298.12±10.05 ^B	287.66±7.76 ^B	285.32±21.01 ^B
	低浓度组 low	298.55±11.26 ^a	312.68±15.98 ^{Bc}	385.46±18.82 ^{Dd}	311.22±15.46 ^{Cb}	308.65±13.33 ^{Cb}	311.66±21.25 ^{Cb}	309.78±18.78 ^{Cb}
	中浓度组 middle	295.66±15.12 ^a	356.78±10.66 ^{Cc}	333.45±9.87 ^{Cb}	331.98±4.98 ^{bb}	329.54±21.21 ^{Db}	333.66±19.12 ^{Db}	321.98±22.63 ^{Db}
	高浓度组 high	292.33±11.12 ^b	198.76±9.89 ^{Aa}	191.22±15.55 ^{Aa}	199.24±12.16 ^{Aa}	196.31±13.56 ^{Aa}	199.21±11.98 ^{Aa}	195.98±15.16 ^{Aa}
过氧化氢酶(U/mg prot) catalase	对照组 control	3.09±0.81	3.08±0.25	3.12±0.33	2.99±0.76	3.05±0.94	2.98±0.24	2.96±0.35
	低浓度组 low	2.99±0.58	3.12±0.72	2.97±0.46	2.92±0.59	2.96±0.81	2.90±0.71	2.96±0.68
	中浓度组 middle	3.15±0.12	2.93±0.98	3.99±0.64	3.52±0.58	3.14±0.32	3.33±0.73	3.36±0.45
	高浓度组 high	2.98±0.53 ^a	3.05±0.46 ^a	3.12±0.38 ^a	2.99±0.65 ^a	3.24±0.25 ^b	3.30±0.78 ^b	3.28±0.98 ^b
丙二醛(nmol/mg prot) malondialdehyde	对照组 control	1.66±0.31 ^a	1.68±0.24 ^{Aa}	1.65±0.11 ^{Aa}	1.71±0.18 ^{Aa}	1.68±0.51 ^{Aa}	1.82±0.31 ^{Ab}	1.88±0.12 ^{Ab}
	低浓度组 low	1.78±0.21 ^a	1.99±0.38 ^{Bb}	2.01±0.66 ^{Bb}	2.05±0.45 ^{Bb}	2.09±0.11 ^{Bb}	2.41±0.32 ^{Bc}	2.55±0.95 ^{Bd}
	中浓度组 middle	1.55±0.01 ^a	2.22±0.15 ^{Cb}	2.21±0.33 ^{Cb}	2.25±0.78 ^{Cb}	2.29±0.16 ^{Cb}	2.31±0.28 ^{Bb}	2.27±0.47 ^{Bb}
	高浓度组 high	1.65±0.59 ^a	3.81±0.78 ^{Db}	3.83±0.32 ^{Db}	3.89±0.13 ^{Db}	3.81±0.82 ^{Db}	3.85±0.68 ^{Cb}	3.81±0.45 ^{Cb}

注: 同列不同大写字母表示氨氮浓度影响显著($P<0.05$), 同行不同小写字母表示胁迫时间影响显著($P<0.05$), 下同

Notes: the different capital letters of the same column values have significant effect of ammonia levels ($P<0.05$), the different lowercase letters superscripts of the same line values have significant effect of stress times ($P<0.05$), the same below

2.2 黄颡鱼HSP70和HSP90基因mRNA的表达

对照组实验鱼肝脏、脑和鳃中HSP70基因的mRNA表达量在各对照组间无显著性差异($P>0.05$); 低、中、高浓度组实验鱼肝脏中HSP70基因的mRNA表达量呈先降低后升高的趋势, 最低

值出现在6 h时($P<0.05$); 低、中、高浓度组实验鱼脑中HSP70基因的mRNA在0 h时表达量最高($P<0.05$); 低、中、高浓度组实验鱼鳃中HSP70基因的mRNA表达量呈持续升高的趋势($P<0.05$)。氨氮胁迫3 h时, 低、中、高浓度组实验鱼肝脏和脑中HSP70基因的mRNA表达量显著

低于对照组，而鳃中HSP70基因的mRNA表达量却显著高于对照组($P<0.05$)(图1)。

对照组实验鱼肝脏、脑和鳃中HSP90基因的mRNA表达量在各对照组间无显著性差异($P>0.05$)；高浓度组实验鱼肝脏和鳃中HSP90基因的mRNA表达量在24 h时达到最高($P<0.05$)；低、中、高浓度组实验鱼脑中HSP90基因的mRNA表达量在各处理组间无显著性差异($P>0.05$)。氨氮胁迫24 h时，高浓度组实验鱼肝脏和鳃中HSP90基因的mRNA表达量显著高于其他组($P>0.05$)(图2)。

3 讨论

养殖生产过程中，水体中氨氮超标，极易造成养殖鱼类大规模死亡，以往的研究提示，氧化应激可能是导致鱼类氨中毒死亡的重要因素之一^[24-26]。氨氮应激会导致鱼体内产生大量的ROS，尽管鱼类能够依靠自身抗氧化酶体系，如超氧化物歧化酶、过氧化物酶及谷胱甘肽过氧化物酶等清除多余的ROS，但清除效率往往会受到胁迫持续时间和胁迫强度的影响。本研究发现，由于毒性效应，低、中浓度组实验鱼肝脏中SOD活性于胁迫6 h内呈短暂升高趋势，但随着毒性的不断增强，SOD活性于6 h后逐渐受到抑制，在高浓度组实验鱼肝脏中，SOD活性在整个胁迫过程中始终呈现显著的下降趋势，这与Li等^[27]报道的高浓度氨氮胁迫下黄颡鱼肝脏中SOD活性逐渐降低的结果是一致的，原因可能与氨氮胁迫过程中有毒物质(如MDA)积累量超过机体的耐受上限有关。本研究还发现，高浓度组实验鱼肝脏中CAT的活性于24 h时显著升高，而SOD活性早在3 h时已发生显著变化，这可能与不同类型的抗氧化酶在组织中的分布差异有关，SOD在肝脏中的分布较CAT多^[28]。此外，Li等^[29]提出，抗氧化酶活性的变化往往受到组织中MDA积累量的影响。在本研究中，实验鱼肝脏中MDA含量在3 h时迅速达到最高，进一步验证了Li等^[29]的发现。这种现象在其他鱼类中也有报道：鲮(*Mugil cephalus*)暴露在3.0 mg/L氨氮浓度下15 d，实验鱼肝脏中MDA含量是对照组的5倍^[30]；高温胁迫下，吉富罗非鱼(*GIFT O. niloticus*)肝脏中MDA含量随着胁迫时间的延长而升高^[31]；黄颡鱼肝脏中MDA含量随着氨氮胁迫时间的延长而呈逐渐升高的趋势^[32]。

HSPs作为避免生物体遭受氧化应激伤害的

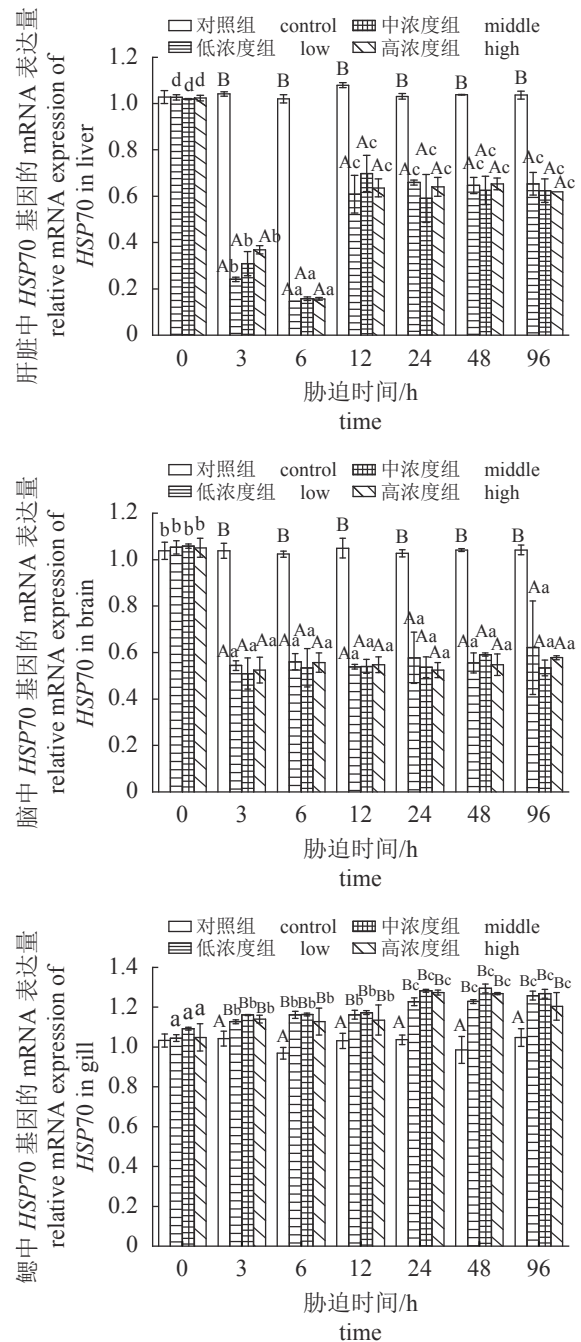


图1 不同氨氮浓度暴露96 h对黄颡鱼肝脏、脑和鳃中HSP70基因mRNA表达水平的影响

不同小写字母表示同一氨氮处理组随胁迫时间影响显著($P<0.05$),不同大写字母表示同一时间处理组之间影响显著($P<0.05$)

Fig. 1 mRNA expression levels of HSP70 in the liver, brain and gills of *P. fulvidraco* during 96 h exposure to different concentration of environmental ammonia

Different lower-case letters indicate that the same ammonia nitrogen treatment group has a significant effect with stress time ($P<0.05$), and different upper-case letters indicate that the effect is significant between the treatment groups at the same time ($P<0.05$), the same below

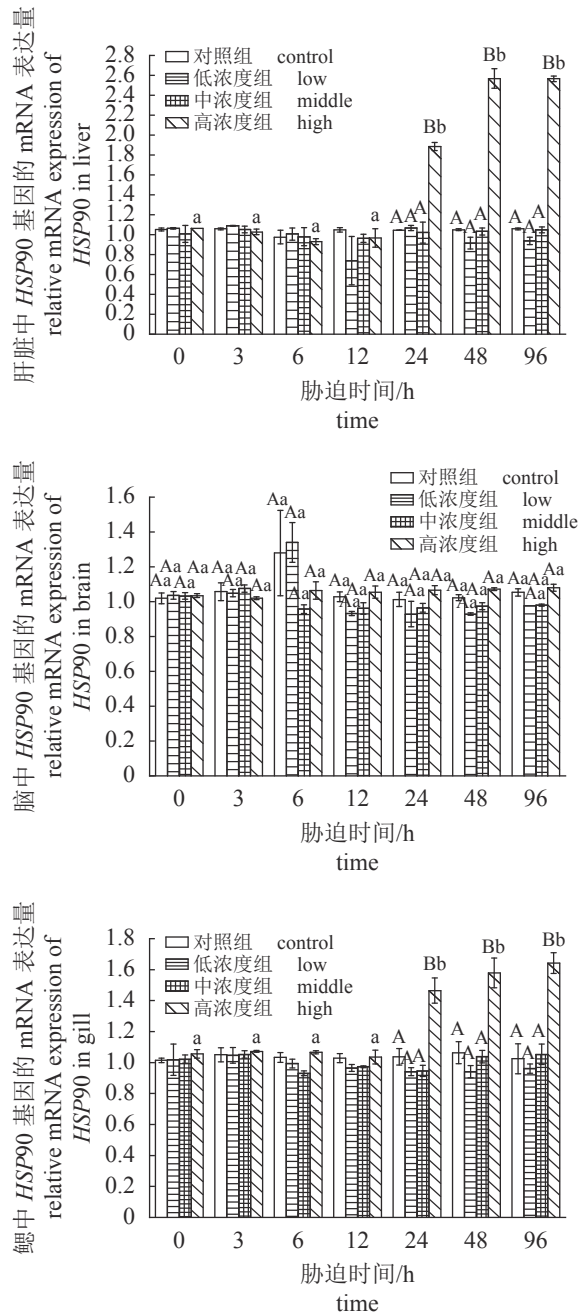


图2 不同氨氮浓度暴露96 h对黄颡鱼肝脏中肝脏、脑和鳃中HSP90基因mRNA表达水平的影响

Fig. 2 mRNA expression levels of HSP90 in the liver, brain and gills of *P. fulvidraco* during 96 h exposure to different concentration of environmental ammonia

一种自我保护系统, 在细胞内具有抗氧化的生物活性, 当暴露于环境应激中, 机体通过激活热休克基因合成HSPs^[33-35]。细胞内HSP70基因表达水平的提高, 可以减少H₂O₂对细胞膜造成的损伤^[36]。本研究发现, 高、中、低氨氮处理组黄颡鱼肝脏中HSP70基因的mRNA表达量均在6 h后

呈现出显著上升趋势, 表明HSP70基因在面对氨氮胁迫时可以快速做出应答。辛苑茹等^[37]对急性高温胁迫下虹鳟幼鱼HSPs基因表达变化的检测中也获得了类似的结果。本研究还发现, 高、中、低浓度组黄颡鱼脑中HSP70基因的mRNA表达量于胁迫发生后均迅速降低, 这是因为血液中过量的NH₃突破了血脑屏障, 对脑组织造成了损伤, 影响到HSP70基因的转录, 这与哺乳类氨中毒引起脑损伤的结果一致^[38]。以往的研究发现, 当环境胁迫发生时, 鱼类鳃组织中HSP70基因的表达量能够在较短的时间内迅速上调^[39-40], 这与本研究中的结果一致: 高、中、低实验组黄颡鱼鳃中HSP70基因的mRNA表达量在3 h时迅速达到最高值, 这种机体的自我保护机制, 能够在很大程度上减少环境应激在短时间内对组织造成的伤害。相对HSP70, HSP90基因对于环境胁迫的发生并不敏感, 只有当应激较为剧烈时才会诱导HSP90基因的转录^[41], 本研究进一步证实了这个现象: 高浓度组黄颡鱼肝脏和鳃中HSP90基因的mRNA表达量于胁迫发生24 h后才出现显著上调。此外, 以往有研究提出, 鱼类脑组织中HSP90表达量更易受到外界环境的影响^[42]。Dietz等^[43]证实, 冬季当温度从18 °C升高到28 °C时, 虾虎鱼脑组织中HSP90合成量增加, 夏季温度上升到32 °C后, HSP90基因才开始表达。然而, 在本研究中, 高、中、低浓度组黄颡鱼脑中HSP90基因的表达量始终没有发生变化, 这可能与胁迫强度或时间有关。

综上所述, 不同浓度的氨氮胁迫会对黄颡鱼抗氧化酶活性造成不同程度的抑制, 原因与丙二醛的积累量有关; 相比HSP90基因, 黄颡鱼HSP70基因的表达量在氨氮胁迫发生后迅速上调, 而这种生理调控机制提示HSP70在应对急性氨氮胁迫时发挥着更重要的作用。

参考文献:

- [1] Jensen F B. Nitrite disrupts multiple physiological functions in aquatic animals[J]. *Comparative Biochemistry and Physiology-Part A: Molecular & Integrative Physiology*, 2003, 135(1): 9-24.
- [2] Kroupova H, Machova J, Svobodova Z. Nitrite influence on fish: a review[J]. *Veterinárni Medicína*, 2005, 50(11): 461-471.
- [3] Aggergaard S, Jensen F B. Cardiovascular changes and

- physiological response during nitrite exposure in rainbow trout[J]. *Journal of Fish Biology*, 2001, 59(1): 13-27.
- [4] Lewis Jr W M, Morris D P. Toxicity of nitrite to fish: a review[J]. *Transactions of the American Fisheries Society*, 1986, 115(2): 183-195.
- [5] Reinehr R, Görg B, Becker S, *et al.* Hypoosmotic swelling and ammonia increase oxidative stress by NADPH oxidase in cultured astrocytes and vital brain slices[J]. *Glia*, 2007, 55(7): 758-771.
- [6] Ip Y K, Tay A S, Lee K H, *et al.* Strategies for surviving high concentrations of environmental ammonia in the swamp eel *Monopterus albus*[J]. *Physiological and Biochemical Zoology*, 2004, 77(3): 390-405.
- [7] Wegele H, Müller L, Buchner J. Hsp70 and Hsp90—a relay team for protein folding[M]. Berlin, Heidelberg: Springer, 2004.
- [8] Feder M E, Hofmann G E. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology[J]. *Annual Review of Physiology*, 1999, 61: 243-282.
- [9] Gething M J. Molecular chaperones: clasping the prize[J]. *Current Biology*, 1996, 6(12): 1573-1576.
- [10] Hartl F U. Molecular chaperones in cellular protein folding[J]. *Nature*, 1996, 381(6583): 571-580.
- [11] Yamamoto Y, Kume M, Yamaoka Y. Implications of heat shock proteins during liver surgery and liver perfusion[M]//Oldhafer K J, Lang H, Pichlmayr R. Isolated Liver Perfusion for Hepatic Tumors. Berlin, Heidelberg: Springer, 1998: 157-172.
- [12] Liu T E, Daniels C K, Cao S S. Comprehensive review on the HSC70 functions, interactions with related molecules and involvement in clinical diseases and therapeutic potential[J]. *Pharmacology & Therapeutics*, 2012, 136(3): 354-374.
- [13] Rutherford S, Knapp J R, Csermely P. Hsp90 and developmental networks[M]//Csermely P, Vigh L. Molecular aspects of the stress response: chaperones, membranes and networks. New York, NY: Springer, 2007: 190-197.
- [14] 李义杰, 张保国. 热休克蛋白的生物学功能及其对细胞的辐射防护作用[J]. *辐射防护通讯*, 2007, 27(6): 31-36.
- Li Y J, Zhang B G. Biological function of heat shock proteins and their radiation protection function for cells[J]. *Radiation Protection Bulletin*, 2007, 27(6): 31-36(in Chinese).
- [15] Cara J B, Aluru N, Moyano F J, *et al.* Food-deprivation induces HSP70 and HSP90 protein expression in larval gilthead sea bream and rainbow trout[J]. *Comparative Biochemistry and Physiology-Part B: Biochemistry and Molecular Biology*, 2005, 142(4): 426-431.
- [16] 刘波, 王美垚, 谢骏, 等. 低温应激对吉富罗非鱼血清生化指标及肝脏HSP70基因表达的影响[J]. *生态学报*, 2011, 31(17): 4866-4873.
- Liu B, Wang M Y, Xie J, *et al.* Effects of acute cold stress on serum biochemical and immune parameters and liver HSP70 gene expression in gift strain of Nile tilapia (*Oreochromis niloticus*)[J]. *Acta Ecologica Sinica*, 2011, 31(17): 4866-4873(in Chinese).
- [17] 周彦静. 持续热应激下虹鳟热应激蛋白基因HSP90 α 和HSP10mRNA表达、组织损伤及血细胞凋亡变化[D]. 兰州: 甘肃农业大学, 2017.
- Zhou Y J. Effects of continuing heat stress on heat-shock protein HSP90 α and HSP10mRNA expression, tissue injury and blood cell apoptosis in rainbow trout (*Oncorhynchus mykiss*)[D]. Lanzhou: Gansu Agricultural University, 2017 (in Chinese).
- [18] 农业农村部渔业渔政管理局, 全国水产技术推广总站, 中国水产学会. 中国渔业统计年鉴2018[M]. 北京: 中国农业出版社, 2018: 25.
- Fishery Bureau of Ministry of Agriculture and Rural Affairs of China. National Fisheries Technology Extension Center, China Society of Fisheries. China fishery statistical yearbook 2018[M]. Beijing: China Agriculture Press, 2018 (in Chinese).
- [19] Hegazi M M, Attia Z I, Ashour O A. Oxidative stress and antioxidant enzymes in liver and white muscle of Nile tilapia juveniles in chronic ammonia exposure[J]. *Aquatic Toxicology*, 2010, 99(2): 118-125.
- [20] Beauchamp C, Fridovich I. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels[J]. *Analytical Biochemistry*, 1971, 44(1): 276-287.
- [21] Aebi H. Catalase *in vitro*[J]. *Methods in Enzymology*, 1984, 105: 121-126.
- [22] Buege J A, Aust S D. Microsomal lipid peroxidation[J]. *Methods in Enzymology*, 1978, 52: 302-310.
- [23] Schmittgen T D, Livak K J. Analyzing real-time PCR
中国水产学会主办 sponsored by China Society of Fisheries

- data by the comparative CT method[J]. *Nature Protocols*, 2008, 3(6): 1101-1108.
- [24] Liang Z X, Liu R, Zhao D P, *et al.* Ammonia exposure induces oxidative stress, endoplasmic reticulum stress and apoptosis in hepatopancreas of pacific white shrimp (*Litopenaeus vannamei*)[J]. *Fish & Shellfish Immunology*, 2016, 54: 523-528.
- [25] Rama S, Manjabhat S N. Protective effect of shrimp carotenoids against ammonia stress in common carp, *Cyprinus carpio*[J]. *Ecotoxicology and Environmental Safety*, 2014, 107: 207-213.
- [26] Cheng C H, Yang F F, Ling R Z, *et al.* Effects of ammonia exposure on apoptosis, oxidative stress and immune response in pufferfish (*Takifugu obscurus*)[J]. *Aquatic Toxicology*, 2015, 164: 61-71.
- [27] Li M, Gong S Y, Li Q, *et al.* Ammonia toxicity induces glutamine accumulation, oxidative stress and immunosuppression in juvenile yellow catfish *Pelteobagrus fulvidraco*[J]. *Comparative Biochemistry and Physiology-Part C: Toxicology & Pharmacology*, 2016, 183-184: 1-6.
- [28] 蒋琦辰, 顾曙余, 张文逸, 等. 氨氮急性胁迫及其毒后恢复对红螯光壳螯虾幼虾相关免疫和代谢指标的影响[J]. *水产学报*, 2013, 37(7): 1066-1072.
- Jiang Q C, Gu S Y, Zhang W Y, *et al.* Acute effects of ammonia exposure on selected immunological and metabolic parameters in juvenile red claw crayfish (*Cherax quadricarinatus*) and the post-exposure recovery[J]. *Journal of Fisheries of China*, 2013, 37(7): 1066-1072(in Chinese).
- [29] Li M, Yu N, Qin J G, *et al.* Effects of ammonia stress, dietary linseed oil and *Edwardsiella ictaluri* challenge on juvenile darkbarbel catfish *Pelteobagrus vachelli*[J]. *Fish & Shellfish Immunology*, 2014, 38(1): 158-165.
- [30] 蒋玫, 李磊, 沈新强, 等. 慢性氨氮胁迫对鲮鱼(*Mugil cephalus*)幼鱼组织细胞免疫指标的影响研究[J]. *海洋与湖沼*, 2014, 45(3): 529-535.
- Jiang M, Li L, Shen X Q, *et al.* Effect of ammonia stress on immunity indicators of juvenile *Mugil cephalus*[J]. *Oceanologia et Limnologia Sinica*, 2014, 45(3): 529-535(in Chinese).
- [31] 强俊, 杨弘, 王辉, 等. 急性温度应激对吉富品系尼罗罗非鱼(*Oreochromis niloticus*)幼鱼生化指标和肝脏HSP70mRNA表达的影响[J]. *海洋与湖沼*, 2012, 43(5): 943-953.
- Qiang J, Yang H, Wang H, *et al.* The effect of acute temperature stress on biochemical indices and expression of liver HSP70 mRNA in gift Nile tilapia juveniles (*Oreochromis niloticus*)[J]. *Oceanologia et Limnologia Sinica*, 2012, 43(5): 943-953(in Chinese).
- [32] 黎庆, 龚诗雁, 黎明. 慢性氨氮暴露诱发黄颡鱼幼鱼谷氨酰胺积累、氧化损伤及免疫抑制的研究[J]. *水产学报*, 2015, 39(5): 728-734.
- Li Q, Gong S Y, Li M. Chronic ammonia toxicity induces glutamine accumulation, oxidative damage and immunosuppression of juvenile yellow catfish *Pelteobagrus fulvidraco*[J]. *Journal of Fisheries of China*, 2015, 39(5): 728-734(in Chinese).
- [33] Parsell D A, Lindquist S. The function of heat-shock proteins in stress tolerance: degradation and reactivation of damaged proteins[J]. *Annual Review of Genetics*, 1993, 27: 437-496.
- [34] Bond U, Schlesinger M J. Heat-shock proteins and development[J]. *Advances in Genetics*, 1987, 24: 1-29.
- [35] Leppä S, Sistonen L. Heat shock response-pathophysiological implications[J]. *Annals of Medicine*, 1997, 29(1): 73-78.
- [36] Gullo C A, Teoh G. Heat shock proteins: to present or not, that is the question[J]. *Immunology Letters*, 2004, 94(1-2): 1-10.
- [37] 辛苑茹, 温海深, 李吉方, 等. 急性高温胁迫对虹鳟二倍体和三倍体幼鱼hsp基因表达的影响[J]. *中国海洋大学学报*, 2019, 49(3): 129-137.
- Xin Y R, Wen H S, Li J F, *et al.* Effects of acute thermal stress on gene expression of heat shock protein in diploid and triploid juvenile rainbow trout (*Oncorhynchus mykiss*)[J]. *Periodical of Ocean University of China*, 2019, 49(3): 129-137(in Chinese).
- [38] Smart G R. Investigations of the toxic mechanisms of ammonia to fish-gas exchange in rainbow trout (*Salmo gairdneri*) exposed to acutely lethal concentrations[J]. *Journal of Fish Biology*, 1978, 12(1): 93-104.
- [39] Deane E E, Woo N Y S. Impact of nitrite exposure on endocrine, osmoregulatory and cytoprotective functions in the marine teleost *Sparus sarba*[J]. *Aquatic Toxicology*, 2007, 82(2): 85-93.
- [40] Zhang A Y, Zhou X F, Wang X Y, *et al.* Characterization of two heat shock proteins

- (Hsp70/Hsc70) from grass carp (*Ctenopharyngodon idella*): evidence for their differential gene expression, protein synthesis and secretion in LPS-challenged peripheral blood lymphocytes[J]. *Comparative Biochemistry and Physiology-Part B: Biochemistry and Molecular Biology*, 2011, 159(2): 109-114.
- [41] Zhang X Y, Zhang M Z, Zheng C J, *et al.* Identification of two hsp90 genes from the marine crab, *Portunus trituberculatus* and their specific expression profiles under different environmental conditions[J]. *Comparative Biochemistry and Physiology-Part C: Toxicology and Pharmacology*, 2009, 150(4): 465-473.
- [42] Tomanek L, Helmuth B. Physiological ecology of rocky intertidal organisms: a synergy of concepts[J]. *Integrative and Comparative Biology*, 2002, 42(4): 771-775.
- [43] Dietz T J, Somero G. The threshold induction temperature of the 90-kDa heat shock protein is subject to acclimatization in eurythermal goby fishes (genus *Gillichthys*)[J]. *Proceedings of the National Academy of Sciences of the United States of America*, 1992, 89(8): 3389-3393.

Effects of acute ammonia stress on antioxidant enzyme activity and mRNA expression levels of *HSP70* and *HSP90* genes in tissues of yellow catfish (*Pelteobagrus fulvidraco*)

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Abstract: This study was carried out to test the effect of acute ammonia stress on antioxidant enzyme activity and mRNA expression levels of *HSP70* and *HSP90* genes in tissues of *Pelteobagrus fulvidraco*, and 360 *P. fulvidraco* [(17.25 ± 0.05) g] were randomly exposed to ammonia: 0 (control), 5.70, 28.50 and 57.00 mg/L total ammonia concentrations for 96 h, respectively. Experimental fish were sampled at 0, 12, 24, 48 and 96 h. The results showed that the superoxide dismutase (SOD) activity in liver of fish in low and medium concentration groups increased from 0 to 6 h, and then gradually decreased, but high concentration group continued to decrease. Malondialdehyde (MDA) content in liver of fish in low, medium and high concentration groups was significantly increased throughout the 96-h period; at hour 3, SOD activity in liver of fish in high concentration group was the lowest, while MDA content was the highest. Liver catalase activity increased significantly at hour 24. In low, medium and high concentration groups, the mRNA expression of *HSP70* in liver of fish decreased from 0 to 6 h, and then gradually increased, and that in gill of fish continued to increase, but that in brain of fish continued to decrease. The mRNA expression of *HSP70* in liver and brain of fish was significantly lower than that of control group at hour 3, but that in gills is opposite. Compared with *HSP70*, the mRNA expression of *HSP90* in liver and gill of fish in high concentration group reached the highest value at hour 24. The results showed that the antioxidant enzymes activity was inhibited by different concentrations of ammonia, and the reason is related to the accumulation of MDA. Compared with *HSP90*, the mRNA expression of *HSP70* in *P. fulvidraco* was rapidly up-regulated after the occurrence of ammonia stress, and this physiological regulation mechanism suggests that *HSP70* plays a more important role in the response to acute ammonia stress.

Key words: *Pelteobagrus fulvidraco*; ammonia nitrogen; antioxidant enzyme; *HSP70*; *HSP90*

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