动物科学

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急性低氧对鲫鱼幼鱼血液基础指标的影响*

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摘要:为考查急性低氧对鲫鱼(*Carassius auratus* L.)幼鱼血液基础指标的影响,将身体健康、体重为(267.26 ± 13.93) g 实验鱼 12 尾随机平均分为两组,对照组置于水温 20 ℃、溶氧浓度为(7.5 ± 0.5) mg · L^{-1} 的水环境中,处理组置于相同水温但溶氧浓度为(1.0 ± 0.2) mg · L^{-1} 的低氧水环境中,两组均进行 2 h 处理;测定实验鱼静脉血 pH 值、红细胞数和血红蛋白浓度,并计算平均红细胞血红蛋白量(*MCH*)。结果显示,处理组的 pH 值为(5.71 ± 0.11),低于对照组的(6.21 ± 0.07);处理组红细胞数为(1.05 ± 0.6) × 10^{12} 个 · L^{-1},血红蛋白浓度为(112.0 ± 6.7) g · L^{-1},均显著高于对照组的相应指标即(0.52 ± 0.04) × 10^{12} 个 · L^{-1} 和(70.9 ± 8.6) g · L^{-1};3 个血液指标在处理组和对照组之间均有显著差异($p < 0.05$);对照组与处理组 *MCH* 值分别为(13.8 ± 1.80)、(10.8 ± 0.63) pg,两者无显著差异。研究提示,急性低氧处理可引起实验鱼血液中红细胞数、血红蛋白浓度增加,pH 值降低,这可能将导致血液载氧和卸氧能力的增强。

关键词:急性低氧;鲫鱼;血液 pH;红细胞;血红蛋白
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溶氧水平降低常常会导致鱼类摄食量减少、生长速度减慢、生殖力下降,甚至引起死亡[1-2]。急性低氧作为鱼类生活过程中不可避免的短期胁迫,对其生存也会产生重要影响。因此,鱼类通过对生存环境的长期适应,获得了很多耐受低氧的机制。鱼类一般可通过提高呼吸频率[3]、增大鳃丝表面积[4]、增强血氧亲和力[5]和提高血液周转率[6]来适应低氧环境。而血氧亲和力与血液中的红细胞数目、血红蛋白含量[7-10]、血红蛋白类型[11-12]、磷酸盐浓度、无机盐离子平衡和氢离子浓度[9,13]有关。

鲫鱼(*Carassius auratus* L.)是世界性分布鱼类,生活于中下层水域,对低氧具有极强的耐受性;目前,研究发现,在低氧状态下鲫鱼鳃丝表面积增加,能量消耗降低,无氧代谢增强[14-17]。因此,本实验研究了急性低氧对鲫鱼血液 pH 值、红细胞数目以及血红蛋白浓度的影响,以期为鱼类生理生化研究提供一些基础数据。

1 材料与方法

1.1 实验鱼的来源和驯化

实验鲫鱼购自合川水产校,于重庆师范大学进化生理与行为学实验室自净化循环控温水槽中驯化 7 d。该水槽规格为 1.2 m × 0.55 m × 0.55 m,实际水容量 250 L,专利申请号为 200520010485.9。实验用水为曝气后的自来水,实验水体温度始终控制在(20.0 ± 1.0) ℃,用充气泵连续向水体充入空气以确保溶氧水平不低于每升 7 mg,日换水量约为驯化水体体积的 10%,光照周期为 12 h 光照:12 h 黑暗。从驯养 10 d 的鲫鱼中挑选身体健康、体重为(267.26 ± 13.93) g 的鲫鱼 12 尾作为实验对象。

1.2 实验方案

将驯养挑选后的实验鱼随机平均分为两组,分别置于氧浓度为(7.5 ± 0.5)、(1.0 ± 0.2) mg · L^{-1} 的水环境中放置 2 h。

采用尾静脉采血法进行血样的采集。将鱼用 30 mg · L^{-1} 的丁香酚麻醉后,用 1 mL 注射器从尾柄侧线处插入取血 0.8 mL。其中 0.3 mL 的血液用作 pH 值测定,通过联接有电极(E-201D 型,上海雷磁仪器厂生产)的酸度计(PHSJ-5 型,上海雷磁仪器厂生产)来进行[18]。

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血红蛋白浓度的测定方法为:用移液管吸取血液20 μL,放入预先盛有4 mL的0.1 mol·L^{-1} NaOH溶液的小试管中,静置15 min后作为测定管。以等量的0.1 mol·L^{-1} NaOH溶液作为空白管,在分光光度计的520 nm处比色。分别测定标准管和测定管的吸光度。计算血红蛋白的浓度公式为

$$\rho = A_{测定管}/A_{标准管} \times 16$$

式中,ρ是100 mL血液中所含血红蛋白克数,$A_{测定管}$是测定管的吸光度,$A_{标准管}$是标准管的吸光度。

测量红细胞数方法参考文献[19],并通过下面的公式计算红细胞数:

每立方毫米血液中的红细胞数 = $n \times 10\ 000$

式中,n为血球计数板上5个中方格的红细胞总数。平均红细胞血红蛋白量(*MCH*)计算公式为

MCH = 每微升血液中血红蛋白浓度 × 10^{12}/每微升血液中红细胞数

1.3 数据统计

采用Excel2000软件对数据进行统计处理,组间比较采用SPSS11.5进行*t*-test分析。统计值均用平均值±标准误(Mean±SE)表示,显著性水平$p < 0.05$。

2 结果

2.1 对血液pH值的影响

经过2 h的急性低氧处理,测得处理组pH值为(5.71±0.11),已显著低于对照组的(6.21±0.07)($p < 0.05$)。

2.2 对红细胞数和血红蛋白浓度的影响

表1显示,低氧处理2 h后,处理组红细胞数和血红蛋白浓度均显著高于对照组($p < 0.05$);其中,处理组红细胞数比对照组增加100.32%,血红蛋白浓度则比对照组增加57.89%。

2.3 对*MCH*值的影响

*MCH*表示每个红细胞内所含蛋白的平均值。表1显示,低氧组*MCH*值较常氧组值略有下降,但差异不显著。

表1 鲫鱼幼鱼红细胞数、血红蛋白浓度和*MCH*

Tab.1 The weight, red blood cell count, hemoglobin concentration and MCH of experimental crucian carp

溶氧水平/(mg·L^{-1})	体重/g	红细胞数目/(10^{12}个·L^{-1})	血红蛋白浓度/(mg·L^{-1})	*MCH*/pg
7.5±0.50	260.19±18.62[a]	0.52±0.04[a]	70.9±8.6[a]	13.8±1.80[a]
1.0±0.20	274.33±22.07[a]	1.05±0.60[b]	112.0±6.7[b]	10.8±0.63[a]

注:同一列上标字母不同的数据间差异显著($p < 0.05$)。

3 讨论

血液载氧量的增加可以通过血液红细胞数增多[20]、血红蛋白浓度增大[21]以及单个红细胞与氧气的结合能力增强来实现。经过30 d低氧驯化的鲤鱼(*Cyprinus carpio*)红细胞数增加7%[22];11~14 d的低氧驯化会使南极鱼(*Pagothenia borchgrevinki*)血红蛋白增加66%[23]。经过12 h的低氧驯化,大盖巨脂鲤(*Colossoma macropomum*)的红细胞数增加约53%,血红蛋白几乎增加1倍[21];本研究发现,鲫鱼幼鱼经过2 h低氧处理使红细胞数增加约100%、血红蛋白浓度增加58%;同上述几种鱼一样,在经历低氧驯化后,鲫鱼幼鱼红细胞数和血红蛋白浓度都会显著增加,以满足机体对氧的需求;不同的是,在两个指标增加幅度相当的情况下,鲫鱼幼鱼驯化所需时间却只有2 h,明显少于上述鱼类,这说明鲫鱼幼鱼的低氧适应过程的反应速度较以上鱼类更快。

当面临低氧环境时,鱼类的血氧亲和力表现出一种权衡:一方面通过增强在鳃部的血氧亲和力,以便更多地装载氧气,另一方面通过降低在组织的血氧亲和力,以便更多地卸载氧气[21]。血液pH值是影响血氧亲和力的一个重要因素;pH升高通常会使血氧亲和力增强,pH降低通常会使血氧亲和力减弱[23]。有研究发现大盖巨脂鲤经过12 h的低氧驯化,pH值由(8.12±0.06)降到(7.66±0.06)[21],这一结果与本研究一致。

血氧亲和力除受血液pH、红细胞数的影响外,还随着ATP、GTP浓度升高而减弱[24-25],随着Mg^{2+}、Ca^{2+}浓度的升高[23]而增强。鲫鱼幼鱼通过急性低氧驯化后,这些因子如何变化,将是进一步研究的方向。

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Animal Sciences

The Effect of Acute Hypoxia on Blood Parameters of Juvenile Crucian Carp

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Abstract: This study concerns the effect of environment acute hypoxia on blood basic parameters of crucian carp (*Carassius auratus* L.) juvenile. Twelve healthy experimental fish with the body weight of (267.26 ± 13.93) g were divided randomly into 2 groups, i.e. fish in the control group were acclimated in water with a 20 ℃ temperature and 7.5 ± 0.5mg · L^{-1} dissolved oxygen level while fish in the acute hypoxia treatment group were acclimated in water with the same temperature but 1.0 ± 0.2mg · L^{-1} dissolved oxygen level for 2h. The venous blood pH, red blood cell counts, hemoglobin concentrationswere measured and mean cell hemoglobin was estimated after 2h experimental treatments at 20 ℃. The results showed that the pH of the hypoxia-treated group(5.71 ± 0.11) was lower than that of the control group (6.21 ± 0.07), whereas the values of both red blood cell counts (1.05 ± 0.6) × 10^{12} individual · L^{-1} and hemoglobin concentrations (112.0 ± 6.7) g · L^{-1} in the hypoxia-treated group were higher than those of the control group, i.e. (0.52 ± 0.04) × 10^{12} individual · L^{-1} for red blood cell counts and (70.9 ± 8.6) g · L^{-1} for hemoglobin concentrations. The three blood parameters between the two experimental groups were all significantly different($p < 0.05$). There was no significant difference in MCH between the two groups. Acute hypoxia treatment induced higher blood red blood cell counts and hemoglobin concentrations but lower blood pH in crucian carp juvenile which might enhance the capacity of blood oxygen loading and unloading capacities

Key words: acute hypoxia; *Carassius auratus* L.; blood pH; red blood cell; hemoglobin

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