

# Effect of Subacute Intoxication with Nitrite on AMP-deaminase Activity in Skeletal Muscles of Common Carp (*Cyprinus carpio*)

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## Abstract

This study was aimed to evaluate the effects of intoxication of common carp with nitrite on some properties of Adenosine 5'-monophosphate (AMP) deaminase in the skeletal muscle. LC<sub>50</sub> value was determined to be 512 mg/L. After estimating the LC<sub>50</sub>, fish were exposed to 153 mg/L (30% LC<sub>50</sub>) and sampling was done at 0, 24, 48, 72, and 96 h after exposure. Cytosolic AMP deaminase was purified from the skeletal muscle of the control and experimental groups. AMP-deaminase showed Hill kinetics with an S<sub>0.5</sub> value for AMP of 2.52±0.031 and 1.45±0.07 mM, a Hill coefficient (n<sub>H</sub>) of 1.35±0.051 and 1.79±0.60, and a maximal velocity (V<sub>max</sub>) of 133.39±28 and 98.9±38 IU/mg protein for the control and experimental groups, respectively after 96 h. The optimum pH was reduced during nitrite intoxication. The maximal activation of the enzyme was at 45°C. Also, activation energy declined in the experimental groups. Fenton reaction showed that the enzyme was tolerant to free radicals.

According to the results, AMP deaminase activity in the common carp's muscles increased following nitrite intoxication. Also, acidic condition increased AMP deaminase activity that could be an adaptive response to hypoxic condition caused by nitrite intoxication.

## Introduction

Nitrite is one of the most common pollutants in water systems, which accumulates by bacterial nitrification of ammonia or by denitrification of nitrate (Martinez & Souza, 2002). Nitrite concentrations are increasing in freshwater ecosystems as a consequence of several anthropogenic sources, such as effluents from industries producing metals, dyes, and celluloids; urban sewage effluents; and aquaculture (Jensen, 2003; Camargo & Alonso, 2006; Zhang et al., 2019). In some eutrophic shallow lakes, an increased concentration of nitrite is usually observed during the degradation of cyanobacterial blooms (Qin et al., 2007; Zhang et al., 2019). Nitrite as an intermediate which produced during

oxidation of ammonium to nitrate causes methemoglobinemia and hypoxia (Russo, 1985; Lewis & Morris, 1986; Eddy & Williams, 1987; Martinez & Souza, 2002; Jensen, 2003; Kroupova et al., 2006; Kroupova et al., 2016; Zhang et al., 2019).

Anoxia tolerance is strengthened by well-developed mechanisms of anoxia-induced metabolic rate depression, high levels of carbohydrate reserves in all tissues, and a novel capacity to avoid acidosis and end product accumulation by processing lactate to form ethanol and CO<sub>2</sub> excreted by the gills (Hochachka & Somero, 1984; Hermes-Lima & Zenteno, 2002; Lushchak, V.I., Lushchak, L.P., Mota, & Hermes-Lima, 2001; Bickler & Buck, 2007; Vornanen, M., Stecyk, J.A.W., & Nilsson, G.E., 2009; Fago & Jensen, 2015 ;

Fagernes et al., 2017; Gattuso et al., 2018)

The first step to compensate for the energy loss in hypoxia is to consume high-energy compounds, such as ATP and phosphocreatine, which can be stored in a limited amount (Hochachka & Somero, 1984; Wang, Y., 2011). Another limitation in providing energy is metabolic acidosis, likely to be caused by the decrease in the pH of the muscle when lactate is produced during glycogen utilization. Thus, during intense exercise, the deamination of AMP to  $\text{NH}_3$  happens via AMP deaminase, adenylate kinase reaction is activated, and the adenylate energy charge is increased (Lushchak, Smirnova, & Storey, 1998; Mandic, M., & Regan, M.D., 2018; Omlin, T., & Weber, J.M., 2010); as a result, the energy charge of the cell is maintained at an acceptable level. AMP deaminase is one of the purine nucleotide cycle enzymes and the key enzyme in preserving the viability of the cell under stressful conditions. Thus, the behavior of AMP deaminase is very important in hypoxic and anoxic conditions which are caused by several factors, including nitrite intoxication. Moreover, this enzyme could contribute to the adaptation of fish to nitrite-induced anoxic conditions.

Cyprinidae family is one of the most diverse bony fish families reared across the world. Common carp (*Cyprinus carpio*) is one of the most resistant species to environmental stresses, especially hypoxia (Svobodova et al., 2005; Lushchak et al., 2005; Bickler & Buck, 2007; Lardon et al., 2013; Moyson, S., 2015; Gattuso, 2018) In recent years, more attention has been paid to the harmful effects of nitrite on fish. Increased nitrite concentrations in water are also one of the most frequent problems encountered in both aquariums and fish farms (Voslarova, Pistekova, Svobodova, & Bedanova, 2008; Kroupova et al., 2010).

In the study of Woo & Chiu (1996) metabolic and osmoregulatory responses of the Sea Bass *Lateolabrax japonicus* to nitrite exposure reviewed. It is postulated that this event probably represents an adaptive strategy employed by the sea bass to counteract nitrite toxicity. Kaminsky & Kosenko (2010) had studied AMP deaminase and adenosine deaminase activities in liver and brain regions in acute ammonia intoxication and subacute toxic hepatitis. They concluded, those are up-regulated under pathological conditions, probably in order to overcome compensatory synthesis of adenylates, to ensure prompt adenylate pool depletion and reduce the adenylate energy charge in liver and selected brain regions.

AMP deaminase has been identified in muscle of several fish species. Nitrite is a natural component of the nitrogen cycle in ecosystems, and its presence in the environment is a potential problem due to its well-documented toxicity (Kroupova, H., Machova, J., & Svobodova, Z., 2005). Our aim was to describe the effects of intoxication of common carp with nitrite on AMP deaminase activity due to its key role in providing energy in these conditions for adaptation.

## Materials and Methods

### Fish Maintenance

Sample common carp (*C. carpio* L.), with a mean weight of  $67.3 \pm 14.7$  gr, were obtained from commercial farms. For two weeks prior to the experimentation, the fish were kept for adaptation to the experimental condition. Water chemical factors were as follows: Temperature, 26-28 °C; Ammonia, 0.01 ppm; nitrite, 0.02 ppm; nitrate, 5 ppm; chloride, 0.5 ppm; total hardness, 180 ppm; pH, 8-8.4; total salinity, 1.2 ppt; and  $\text{O}_2$ , 8 ppm. The fish were fed twice daily with commercial concentrate food (2% of body weight). During this period, 20% of the volume of water was replaced every 48h. All procedures were done as approved by ethics committee of Shahid Chamran University of Ahvaz.

### LC<sub>50</sub> Calculation

Two hundred and ten Common carp were prepared from commercial provider and divided randomly in tanks (n=10) The fish were exposed to different concentrations of nitrite, including 0, 450, 500, 530, 550, 560, and 600 mg/L. Each treatment was done in triplicate. Then, the percentage of mortalities was recorded in the 96 h group. LC<sub>50</sub> in 96 h was estimated using Probit software. LC<sub>50</sub> value was calculated to be 512 mg/L.

### Experimental Design

For sub-lethal concentration, we used 30% of the concentration of the estimated LC<sub>50</sub> (153 mg/L which was used as Sodium nitrite: 230 mg/L). Ninety healthy fish were divided 6 tanks (n=15). Fish divided to nitrite exposed and control group, each in triplicate. Sampling was done at 0 (control group), 24, 48, 72, and 96 h after exposure to nitrite. At each sampling time 3 fish was captured from each group. For this, fish were anesthetized with 2-phenoxyethanol, then fish were killed by trans-spinal dissection, and the muscles (~ 5 g) of the fish was taken from the dorsal region. All procedures were done as approved by ethic committee of the Shahid Chamran University of Ahvaz.

### AMP-deaminase Purification

White skeletal muscles were washed in normal saline and homogenized in homogenate buffer containing 80 mM KCl, 2 mM EDTA, 54 mM  $\text{KH}_2\text{PO}_4$ , 35 mM  $\text{K}_2\text{HPO}_4$ , and pH 6.5 with a few crystals of PMSF (Sigma Aldrich Chemical Co. USA.) to inhibit protease. The homogenate was centrifuged at 12,000 g for 10 min (Eppendorf, Germany), and the supernatant as a crude extract was removed. Enzyme purification was performed according to affinity chromatography with some modifications in the Smiley method (1967). Phosphocellulose (Sigma Aldrich Chemical Co. USA.), as

a resin, was kept 24 h at 4°C for equilibration; then, it was added to the supernatant and the suspension was stirred for 2 h on ice. This made the enzyme to attach to phosphocellulose. Again, the slurry was centrifuged at 12,000 g for 10 min and the supernatant was discarded. To delete the remained proteins, the pellet was washed with homogenate buffer. Finally, the slurry was poured into a column and washed with a progressive concentration of KCl (Sigma Aldrich Chemical Co. USA.) (0.4, 1, 1.5, 2 M, pH 7), which facilitated the binding between the enzyme and AMP. Finally, AMP deaminase was purified with 0.4 M KCl.

### SDS-polyacrylamide Gel Electrophoresis

SDS-polyacrylamide gel electrophoresis was performed according to the method suggested by Laemmli (1970). Thus, 10% separating and 5% acrylamide (Cinnagen, Iran) stacking gels were used and they were stained with Coomassie Brilliant Blue R-250 (Merck, Germany).

### Enzyme Assays

AMPD activity was measured using a Microplate spectrophotometer (Power Wave Xs2, Biotek) at 285 nm for 5 minutes. The difference in the molar absorption of AMP and IMP at 285 nm is 300 (Smiley, Berry, & Suelter, 1967). The reaction volume of 200  $\mu$ l contained 50 mM imidazole-HCl (Merck, Germany) (pH 7), 2 mM AMP (Sigma Aldrich Chemical Co. USA), and 100 mM KCl. Also, the protein concentration was measured by the Bradford method with Coomassie brilliant blue G-250 (Merck, Germany) and Bovine serum albumin (Merck, Germany) as a standard (Bradford, 1976).

To determine the relationships between the effects of pH and the AMP deaminase activity, a wide range of pH (4 to 9), which contained 25 mM imidazole-HCl and Tris-HCl Buffer (Cinnagen, Iran) with pH=7, were used. To evaluate the effects of temperature on AMP deaminase activity, the enzyme activity was assayed at 4, 10, 15, 20, 37, 45 °C in an assay buffer.

### Fenton Reaction

A Fenton system was designed for evaluating the effects of reactive oxygen species on enzyme tolerance. The volume reaction contained assay buffer, 100  $\mu$ M H<sub>2</sub>O<sub>2</sub> and 5  $\mu$ M FeSO<sub>4</sub> prepared at a potassium buffer with pH=7 (Lushchak, Husak, & Storey, 2008).

### Kinetic Measurement

The activity of AMPD of white skeletal muscle of common carp was evaluated daily to determine the half-life of the enzyme. The half-life of the enzyme was defined as the day that the enzyme activity was reduced to half (50% enzyme activity). Kinetic studies ( $K_m$ ,  $V_{max}$ ) were done at various concentrations of the substrate (0, 0.5, 1, 1.5, 2, 2.5, 3, 4, and 5 mM) and in an assay buffer using a nonlinear regression kinetic software.

### Statistical Analysis

The normality of the data was examined using the Kolmogorov–Smirnov test. Data analysis was performed using one-way ANOVA to determine the significant difference between the treatments at  $P \leq 0.05$ . If a significant difference was found, Tukey's multi-domain test post-test was used to compare the results. The statistical analysis of the data was done using SPSS 20 (SPSS for Windows, version 20).

### Results

#### LC50

According to the mortality recorded following 96 h exposure to different levels of sodium nitrite (Figure 1). Mortality and behavioral changes were observed during 96 h. Finally, LC50– 96 h of nitrite was estimated at 512 mg/L. by Probit. The maximum acceptable concentration (MAC) of nitrite was determined 51/24 mg/L.

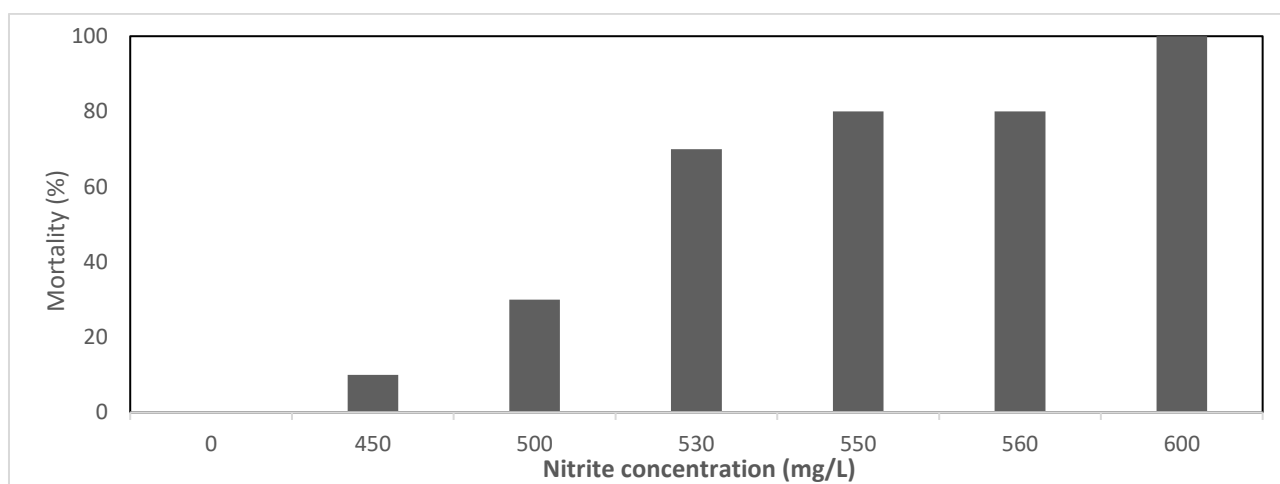


Figure 1. Mortality rate of Common carp exposed to different nitrite concentrations after 96 h

### Purification and Molecular Mass Evaluation

The SDS-PAGE showed that the enzyme was electrophoretically homogenous, with single band and molecular mass of AMPD was estimated to be 55 kDa (Figure 2). The specific activity of the purified enzyme was 1200-1300 U/mg protein.

### Kinetics

Table 1 shows the kinetic properties of AMP-deaminase ( $K_m$ ,  $V_{max}$ , and  $n_H$ ). The data indicated that hypoxia due to intoxication with nitrite modified all kinetic parameters. The substrate affinity data showed a Hill plot ( $P < 0.05$ ), and showed cooperative binding of AMP, that increased in treatment groups.

In experimental groups, the enzyme affinity for the substrate and  $V_{max}$  reduced in comparison to those of the control group ( $P < 0.05$ ). For example, in treatment 96 h,  $S_{0.5}$  and  $V_{max}$  increased by 1.7- and 1.3-fold, respectively. A higher  $S_{0.5}$  means that less of the substrate is required to reach half of  $V_{max}$ . With increasing nitrite intoxication,  $S_{0.5}$  levels decreased in the treatment groups.

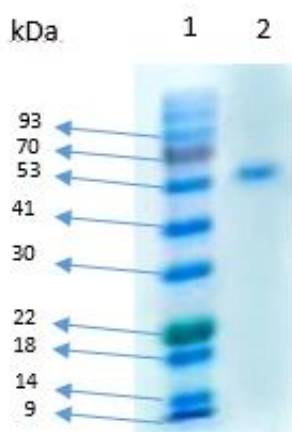
### pH-dependency

Figure 3 illustrates the effects of pH 4 to 9 on the activity of AMP-deaminase. The highest enzyme activity ( $P < 0.05$ ) in the control group was at pH 7, but the optimum pH (pH 6.5) was observed in the experimental groups that were exposed to nitrite and hypoxia. Also,

30% and 50% reduction in the activity of AMP deaminase was recorded at pH 6 and 7, respectively. Experimental groups showed a similar pattern, with the optimum pH felled from 7 to 6.5. Optimum pH in experimental groups was slightly more acidic. The effect of intoxication in causing a acidic shift in pH optimum is observed. On both sides of the optimum pH, the enzyme activity decreased with a steep slope.

### Oxidative Stress

One of the most important sources of reactive oxygen species (ROS) in biological systems is the Fenton reaction. The time needed to complete a Fenton reaction will depend on the many variables, most notably catalyst dose and wastewater strength. For typical reaction times are 30 - 60 minutes. For more complex or more concentrated wastes, the reaction may take several hours (Zhang, 2005). When the Fenton is completed, the activity of the enzyme is compared. The enzyme activity was evaluated in 10, 20, 30, 40, 50, and 60 min after the start of the reaction. The effects of any part of the reaction ( $Fe^{2+}$ ,  $H_2O_2$ ), separately or in combination with other parts, were calculated.  $FeSO_4$  did not affect AMPD activity in the control group. In this, the  $Fe^{2+}$  or  $Fe^{3+}$  without  $H_2O_2$  didn't produce ROS and AMPD activity remained constant, while the AMPD activity in the control group reached half-maximal activity after 50 min by using  $H_2O_2$  in the reaction. Inactivation of AMPD had a slowly decreasing. The combination of  $FeSO_4$  and  $H_2O_2$  reduced sharply the



**Figure 2.** Determination of the molecular weight of AMPD (treatment 96h) by SDS-PAGE. Lane 2 shows the position of AMP deaminase and lane 1 shows the standard proteins.

**Table 1.** The Kinetic properties of AMP-deaminase from white skeletal muscle of Common carp in control group and comparison with treatment groups. Data are expressed as means  $\pm$  S.D., Significantly different ( $P < 0.05$ ),  $n=3$ . The star indicates a significant difference in compare to the control group

	$V_{max}$	$S_{0.5}$ (mM)	$n_H$ (I.U./mg protein)
Control	13.39 $\pm$ 0.28	2.52 $\pm$ 0.03	1.35 $\pm$ 0.05
24 h	11.80 $\pm$ 1.28*	2.19 $\pm$ 0.09*	1.67 $\pm$ 0.08*
48 h	8.21 $\pm$ 0.43*	1.82 $\pm$ 0.10*	1.94 $\pm$ 0.06*
72 h	7.87 $\pm$ 0.19*	1.75 $\pm$ 0.16*	1.59 $\pm$ 0.60*
96 h	9.89 $\pm$ 0.38*	1.45 $\pm$ 0.07*	1.79 $\pm$ 0.60*

enzyme activity by 50% in the control group after 20 min. Similar results were recorded in the 96 h group (Figure 4).

### Half-life

To obtain enzyme stability at 4°C, the enzyme activity was measured in an assay buffer daily. Intoxication with nitrite decreased the enzyme activity and stability over time in the treatment groups. The half-life ( $t_{1/2}$ ) of an enzyme is the time it takes for the activity to reduce to a half of the original activity (Chaplin, M, 2004). The half-life for the control and experimental groups was 7.5 and 6 days, respectively (Figure 5).

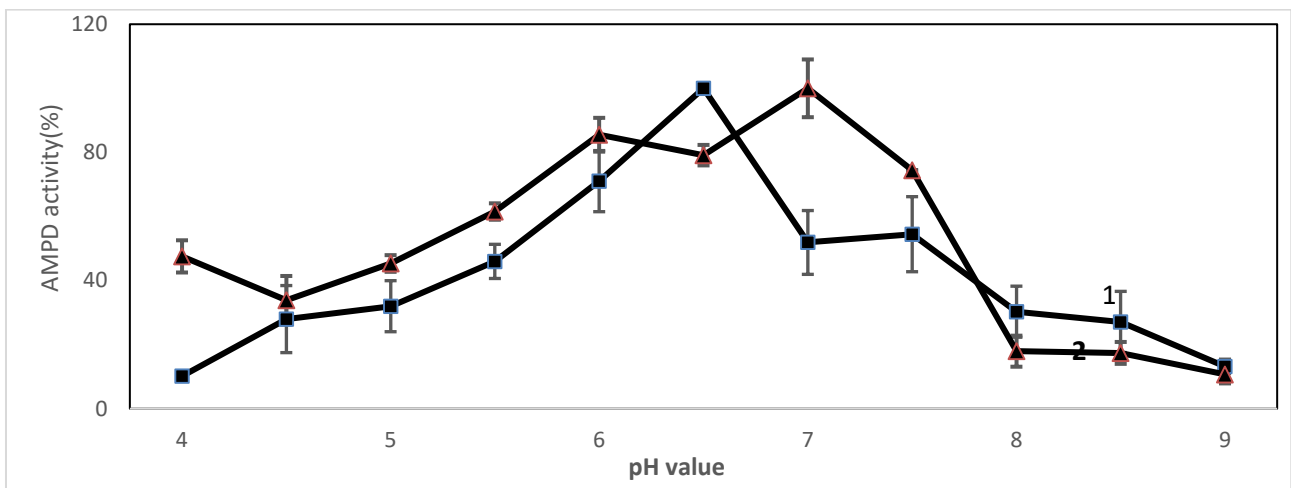
### Urea Inhibition

Half-maximal inhibition ( $I_{50}$  value) in the control

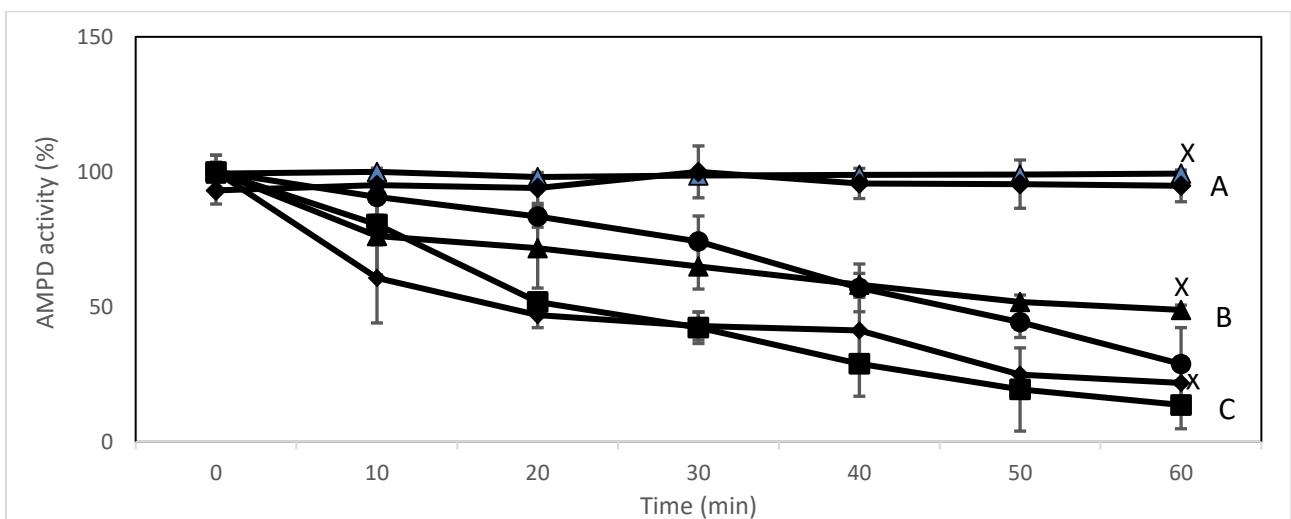
group was 0.07 mM, while it increased to 0.15 mM in the treatment 96 h group ( $P < 0.0001$ , Figure 6). According to the enzyme activity in experimental group, the slope decreased more slowly than in the control group, meaning that higher concentrations of urea than control were required to reduce the enzyme activity by 50%.

### Temperature and Activation Energy

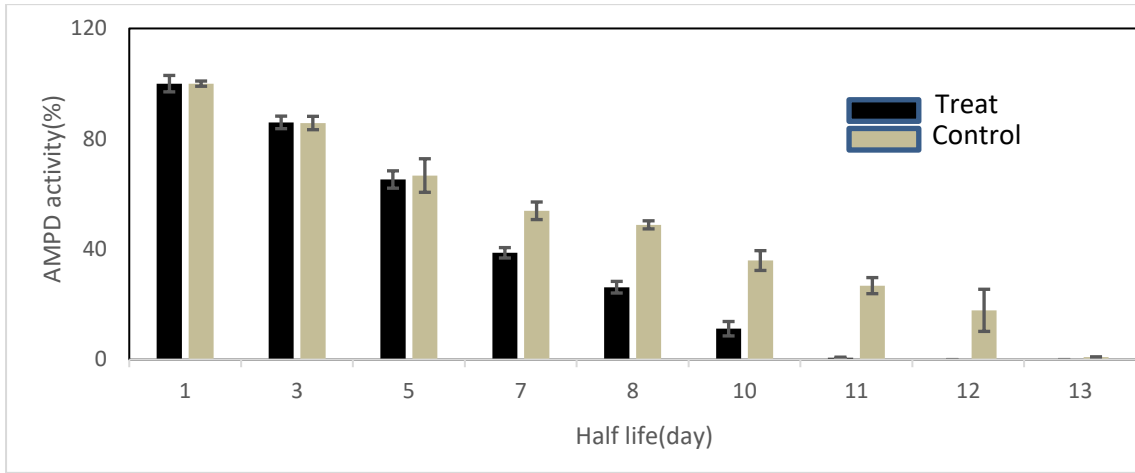
The influences of temperature on AMPD activity is shown in Figure 7. The maximal activity was seen at 45°C. For high temperatures (37°C), the activation energy for the control group was  $18.84 \pm 1.40$  kcal/mol while it was  $9.685 \pm 0.67$  kcal/mol for the 96 h group (Figure 7). The activation energy for temperatures below 37°C for control and nitrite exposed groups were  $21.74 \pm 0.54$  and  $11.36 \pm 0.89$  kcal/mol, respectively.



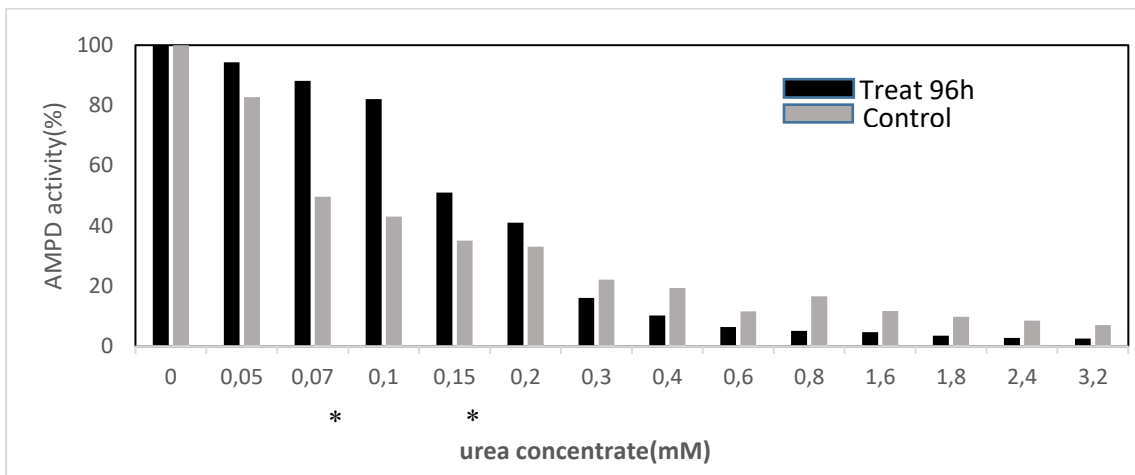
**Figure 3.** The effects of pH on the AMPD activity in muscles of Common carp in the control (1) and the experimental groups (2). Assay conditions: pH 7.0, 25 mM imidazole-HCl buffer, 25 mM Tris-HCl Buffer, adenosine monophosphate 2 mM, KCl 150 mM, 25°C,  $P < 0.05$  and  $n=3$ .



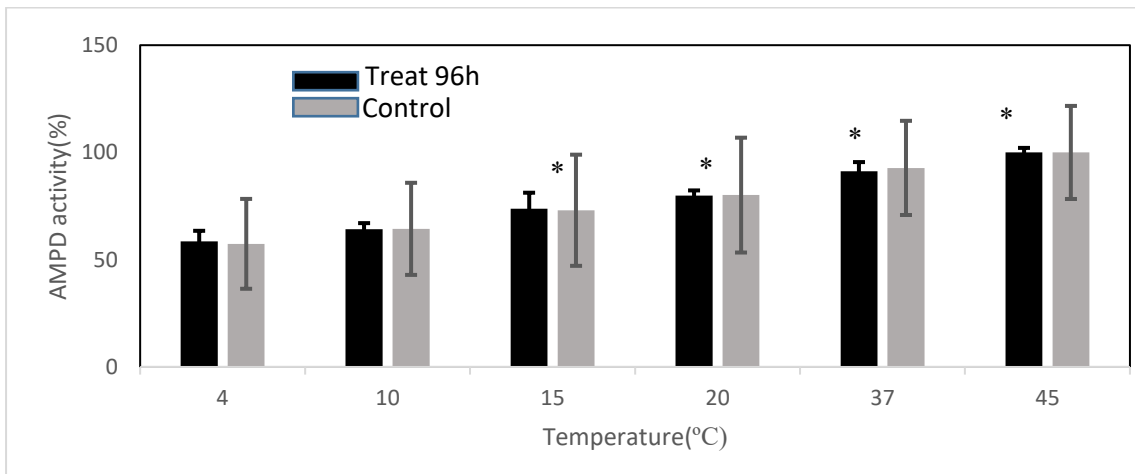
**Figure 4.** The effects of iron ions, the reagent  $\text{FeSO}_4$  concentration was 5  $\mu\text{M}$ , (A). Hydrogen peroxide,  $\text{H}_2\text{O}_2$  concentration was 100  $\mu\text{M}$ , (B). And  $\text{FeSO}_4 + \text{H}_2\text{O}_2$ , the reagent concentration was the same as above mentioned (C); On AMPD activity in control (\*) and treat 96h groups at 25°C,  $P > 0.05$  and  $n=3$ .



**Figure 5.** The relationship between the enzyme activity and stability over time in samples exposed to nitrite for 96 h and the control group,  $P < 0.001$  and  $n = 3$ .



**Figure 6.** The effects of different concentrations of urea on AMPD activity (%) after 96 h. The star indicates a significant difference in compare to the control group ( $P < 0.001$ ).



**Figure 7.** The effect of temperatures (4, 10, 15, 20, 37, and 45°C) on AMPD activity (%) in samples exposed to nitrite for 96 h and the control group,  $P > 0.05$  and  $n = 3$ . The star indicates a significant difference in compare to the control group.

## Discussion

Unfortunately, the removal of industrial pollution from water sources seems to be unachievable, so rearing tolerant species in polluted water helps to compensate for this unfavorable situation. Nitrite accumulation results in the oxidation of hemoglobin and disruption of oxygen transmission. Response to hypoxia requires energy that is supplied through two pathways: phosphagens (ATP and phosphocreatine) and glycolysis. ATP utilization results in a drop in the concentrations of phosphagens and an increase in the AMP and ADP levels (Hochachka & Somero, 1984; Korzeniewski, B, 2005; Nimmo & Ekblom, 2007; Valberg, S.J, 2008). The present study showed that AMP deaminase activity was strongly affected by pH (Figure 3). In the experimental groups and control, the maximal activity was observed at pH 6.5 and 7, respectively. At pH 6, the activity of AMPD in the experimental group decreased by 1.2-fold compared to that of the control group. With the increase in the pH to 6.5 and 7 in the experimental and control group, the activity of AMPD was recorded to decrease by 2- and 1.2-fold, respectively; this finding is similar to those of the previous reports (Lushchak et al., 1998, Korzeniewski, B, 2005; Lushchak, 2008). The discrepancy between the maximum activity in various pH concentrations shows that the adaptation occurred. PH fell in both toxicity and hypoxia due to the anaerobic glycolysis, so AMPD was activated at lower pH concentrations. This finding suggests that the active site of the enzyme may contain an ionized group which can easily be protonated and deprotonated under these conditions. Evidence supporting this notion is provided by Lushchak et al. (2008, 1998).  $S_{0.5}$  for AMP in common carp rose with an increase in the pH (Lushchak et al., 2008), but an opposite pattern was found in the sea scorpion (Lushchak et al., 1998).

The Hill coefficient was higher than 1 in all groups ( $P < 0.05$ ), showing that the subunits of AMP deaminase were an allosteric enzyme, which cooperated with the substrate in the process of binding. The  $V_{max}$  reduction ( $P < 0.05$ ) in the experimental groups in comparison with that of the control indicated that the enzyme saturated at a lower concentration of the substrate. At acidic pH, the activity of the enzyme increased ( $P < 0.05$ ) by the protonation of the substrate-binding site of AMP-deaminase, which increased the enzyme-substrate affinity and accelerated the reduction of  $S_{0.5}$  ( $P < 0.05$ ). The reduction in the  $S_{0.5}$  occurred in groups that had been exposed to nitrite, leading to the activation of AMP deaminase and an increase in the IMP and ammonia.

In the face of stressful conditions, during which the pH decreases and AMP is accumulated, the body needs energy to preserve the viability of cells. AMPD is a sensor of the energy demands of the cells. AMP deaminase catalyzes the hydrolysis AMP to IMP and  $NH_3$  and is a key element in maintaining cellular energy (Sabina & Holmes, 1990; Lushchak et al., 2008, Hancock, 2006; Kosenko & Kaminsky, 2010).

Increased enzyme activity induces purine nucleotide cycle activity, which closely parallels the enhancement of oxidative phosphorylation pathway and the increase in the fumarate production (Mommensen & Hochachka, 1988; Schulte, Moyes, & Hochachka, 1992; Lushchak & Storey, 1994a; Lowenstein, 1972; Ohlendieck, 2010), or provides the cells with energy by anaerobic glycolysis by using malate-aspartate shuttle and converting fumarate to malate.

The high nitrite  $LC_{50}$  and enzyme half-life suggesting that the common carp have low susceptibility to high concentration of nitrite in water (Figure 5), so it could be concluded that Common carp could overcome the results of increased content of nitrite in water such as hypoxia. Similar to our findings, Lushchak et al. (2005) reported that goldfish, *Carassius auratus*, could survive in hypoxic and anoxic conditions. Common carp can successfully survive in hypoxic, anoxic, and hyperoxic environments and is highly tolerant of stress caused by free radicals (Storey, 1996; Lushchak & Bagnyukova, 2006). Nitrite exposure in aquatic ecosystems can induce the formation of ROS and RNS in organisms, which then affects the antioxidant system and other macromolecules (Jensen et al., 2015; Jensen and Hansen, 2011; Sun et al., 2014). In addition, Nitrite exposure oxidizes hemoglobin in hemocytes, increases the amount of methemoglobin, and then causes hypoxia in organisms, which may cause overproduction of ROS (Cheng et al., 2016; Meinelt et al., 2010). As reported in other species, Fenton reaction can be helpful in elucidating adaptation to these environments (Lushchak et al., 1998).

We analyzed the separate effect of any part of the system.  $FeSO_4$  did not affect the AMPD activity in either of the groups ( $P > 0.05$ , Figure 4A). Iron ions present in the assay buffer and muscle extracts had no effect on the AMPD activity. To form the Fenton system to produce Hydroxyl radicals, in addition to iron, hydrogen peroxide is also needed. Since hydrogen peroxide is not present in the environment that was not activated the Fenton system. However, when the AMPD activity was assayed in volume reaction with  $H_2O_2$ , it reached half-maximal activity after  $\sim 50$  min in the control and the treat 96h. This reduction may be due to the Fenton created by iron ions present in the environment (Figure 4B). While a combination of  $FeSO_4$  and  $H_2O_2$  was used, the enzyme activity was reduced for 50% after 20 min in the control group; almost the same result was observed in the 96h group. No significant difference was recorded between the control and experimental groups 30 min after the start of the reaction ( $P > 0.05$ , Figure 4C).

Therefore, our finding shows two aspects compatibility: approximately, no significant differences between control and experimental groups recorded that suggesting enzyme compatibility to free radicals in experimental groups and or free radical with inactivation of AMPD increases its protection effect by increase adenosine production and blood supply to the muscle.

The  $I_{50}$  for AMP was higher in the experimental groups than that of the control group ( $P < 0.0001$ ). This means that the enzyme from the muscle of the fish was inhibited 50% by more urea value and that is another reason for the high adaptation observed in common carp. The molecular mass of AMPD is different from that of other species, such as human uterine 37 kDa (Nagel-Starczynowska, Nowak, & Kaletha, 1991), chick muscle 69 kDa (Sammons & Chilson, 1978), trout muscle 71.6 kDa (Lushchak & Storey, 1994b), and equine muscle 76 kDa (Raffin & Thebault, 1991), and common carp muscle 55 kDa (Lushchak et al., 2008). The variation in the AMPD molecular mass could be due to the limited proteolysis, degradation, interspecies or intertissue specificity, and cross-link during purification.

The calculated activity for the enzyme increased at higher temperatures; this heightened activity actually caused enzyme activation, which is beneficial to enzyme function. The increased enzyme activity could be correlated with 2 potential contribute to binding, hydrogen and hydrophobic binding. At high assay temperatures, it tends to stabilize hydrophobic interactions and activate the enzyme. Also, the activation energy decreased by 1.9-fold in the experimental group, indicating that lower energy is required for reaching the transition state and removing AMP as the energy source.

There are strong evidences that during nitrite intoxication, AMP deaminase activity increase like other enzymes included in energy metabolism such as glycogen phosphorylase and phosphofructokinase (Storey, 1991, Martinez, M.L, 2006). Also hypoxia caused by nitrite intoxication decrease pH by producing lactate during anaerobic glycolysis (Hochachka & Somero, 1984; Nimmo & Ekblom, 2007). The results of our experiment showed that AMP deaminase was activated at lower pH, resistant to radicals caused by the Fenton system and Also urea toxicity increased. All of these changes indicated adaptation to stressful conditions that suggesting when Common carp exposed to nitrite toxicity, this enzyme is responsible for maintaining the energy level It could be concluded that Common carp overcomes situation induced by nitrite intoxication by altering the activity of AMP deaminase.

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