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Effect of dietary tryptophan and betaine on tolerance of Caspian roach (*Rutilus rutilus caspicus*) to copper toxicity

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Abstract: The present study investigated effects of dietary tryptophan (TRP) and betaine (BET) on copper (Cu) toxicity tolerance in the Caspian roach (*Rutilus rutilus caspicus*). The Caspian roach fingerlings were fed diets containing 0, 0.25 and 0.5% TRP or 0, 0.5 and 1 % BET and combination of TRP and BET. Specimens were exposed to Cu (0.8 ppm) after either 30 or 60 days feeding. Mortality was recorded in each treatment 48 h after the Cu exposure. TRP decreased significantly fish mortality. However, BET had no significant effect on fish mortality. Specimens fed on the diet supplemented with 0.25% TRP had the lowest mortality among the treatments. It is concluded that 0.25% TRP reduces mortality of Caspian roach during Cu exposure.

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Introduction

Copper (Cu) is an essential microelement for living organism; however, its higher concentrations are very toxic to aquatic life. Cu naturally exists in all aquatic environments, however, multiple anthropogenic activities may result in elevated concentrations, increased exposure, and potential toxicity to aquatic organisms (Grosell et al., 2007). Cu may be introduced into water bodies from industrial waste or through the use of copper sulfate as therapeutic or algaecide. Cu toxic effects in fish are related to gill damage and hydromineral imbalance, gas exchange disturbance (Adhikari, 2003; Evans et al., 2005, Grosell et al., 2007), stress response (Flik et al., 2002; Fırat et al., 2011; Hoseini and Hosseini, 2012) and oxidative damages (Roméo et al., 2000).

Previous studies revealed the Cu contamination of the Caspian Sea (Agah et al., 2006; Abtahi et al., 2007; Taghipour and Azizi, 2011). Caspian roach is one of the valuable species inhabits in Caspian Sea. Recently, the roach population decreased in the Caspian Sea due to over fishing, degraded habitat and pollution (Hoseini and Jafar Nodeh, 2012). For restocking purpose, the Caspian roach fries and fingerlings (0.5-3 g in weight) are released into the rivers associated with the Caspian Sea. Caspian roach may challenge with Cu toxicity, when it is released into the Caspian Sea. Thus it would be of interest to increase its resistance to Cu intoxication.

Nutritional manipulation is a useful means for increasing fish tolerance to Cu toxicity (Hoseini and Hosseini, 2012). Dietary tryptophan (TRP) can suppress the toxic effects of Cu (Hoseini and Hosseini, 2012). TRP is precursor of serotonin (5-hydroxytryptamine, 5-HT) with a stress-relieving effect (Lepage et al., 2002; Hseu et al., 2003; Hosseini and Hoseini, 2013). Lepage et al. (2003) showed that the brain levels of 5-HT is accompanied with an increase in TRP intake whereas De Boeck et al. (1995) found that the brain levels of 5-HT decreased as a result of Cu exposure in common carp

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(*Cyprinus carpio*) suggesting the involvement of 5-HT in Cu toxicity. On the other hand, previous studies (Lepage et al., 2002; Tejpal et al., 2009; Hoseini and Hosseini, 2010) showed stress mitigation as a result of dietary TRP in different fish species. Christen et al. (1990), Weiss et al. (2002) and Reyes-Gonzales et al. (2009) reported antioxidant properties of TRP. Betaine (BET) is an important compound in living organisms with two main biological roles: methyl donation and osmoprotection. It protects intracellular enzymes against osmotically- or temperature-induced inactivation (Yancey et al., 1982). Also, it has been shown that BET may mitigate stress response related to endosulfan exposure in *Labeo ruhia* (Kumar et al., 2012). Considering the aforementioned roles of TRP and BET, these two compounds can increase the tolerance to Cu intoxication in the Caspian roach. Thus, the aim of the present study was to investigate effect of dietary TRP and BET on survival of Caspian roach exposed to water Cu.

Materials and methods

Fish and maintenance conditions: A total of 1080 fish (1.91± 0.01 g) were randomly distributed among 18 tanks (400 L). Fish were fed control diet (Table 1) for 2 weeks to acclimatize the experimental conditions. Thereafter, the experimental tanks were assigned to nine treatments (each had two tanks). The treatments were fed diets supplemented with TRP and BET as follow (amount in percent of diet): TRP = 0-BET= 0 (control), TRP = 0-BET = 0.5 (TRP0-BET0.5), TRP = 0-BET = 1 (TRP0-BET1), TRP = 0.25 –BET = 0 (TRP0.25-BET0), TRP = 0.25-BET = 0.5 (TRP0.25-BET0.5), TRP = 0.25-BET = 1 (TRP0.25-BET1), TRP = 0.5-BET = 0 (TRP0.5-BET0), TRP = 0.5-BET = 0.5 (TRP0.5-BET0.5) and TRP = 0.5-BET = 1 (TRP0.5-BET1). The specimens were fed three times a day, based on 4% of fish wet weight. All tanks were continuously aerated throughout the trial. Water was replaced at a rate of 60%, every day. Water temperature, pH, dissolved oxygen and total hardness were measured weekly while were 23±1 ºC, 7.7±0.1, > 6 ppm and 180 ppm (CaCO3), respectively. Dissolved oxygen and pH were measured using portable multi-

Table 1. Control diet composition.

Ingredients	%
Fish meal	29
Soybean meal	32.9
Wheat meal	14
Barely meal	14
Fish oil	5
Phytase	0.1
Mineral mix	1
Vitamin mix	1
Methionine	1
Lysine	1
D.C.P	1
Proximate composition	%
Moisture	14
Crude protein	33.9
Crude lipid	7
Ash	4.99

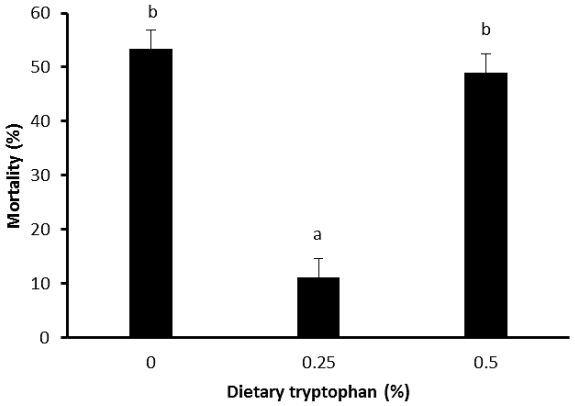

Figure 1. Copper-induced mortality in the Caspian roach fed with diet containing different levels of tryptophan, over 30 d. Different letters show significant difference among the treatments.

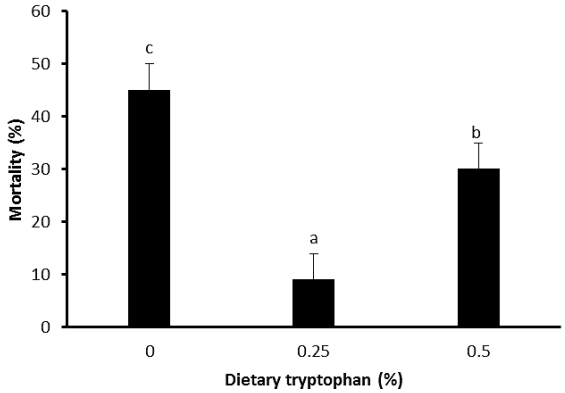

Figure 2. Copper-induced mortality in the Caspian roach fed with diet containing different levels of tryptophan, over 60 d. Different letters show significant difference among the treatments.

Table 2. Two-way ANOVA for survival of the Caspian roach exposed to water copper and fed with tryptophan- and betaine-supplemented diet, over 30 d.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	114	8	14.25	12.41	< 0.0001
Intercept	385.3	1	385.33	335.6	< 0.0001
TRP	96.8	2	48.44	42.19	< 0.0001
BET	4.66	2	2.33	2.03	0.16
TRP × BET	12.44	4	3.11	2.70	0.063
Error	20.66	18	1.14		
Total	520	27			
Corrected Total	134.6	26			

R Squared = 0.847 (Adjusted R Squared = 0.778)

parameter meter (sensION 156, USA). Water total hardness was measured using portable photometer with commercial kits provided by the manufacturer (Wagtecch Portable Photometer 7100, Berkshire, UK).

Copper toxicity test: After 30 and 60 days of feeding, fish were exposed to 0.8 ppm Cu (as copper sulfate) and survival rate was recorded after 48 h. To expose fish to Cu, 30 fish were removed from each treatment and placed in three plastic tanks (20 L). All tanks were aerated continuously. Cu was added to the tanks after a stock solution preparation. Fish were not fed during the Cu exposure. Mortality was checked every 6 h until 48 h. Dead fish were removed immediately.

Statistical analyses: Data were analyzed using two way ANOVA and Duncan test to examine the effects of TRP and BET on survival, at each time, separately. Data are presented as mean ± SE. $P<0.05$ considered as significant difference. All analyses were performed using statistical software SPSS 18.

Results

There was no mortality during the feeding trials. Results showed that fish survival during Cu exposure was significantly ($P<0.0001$) affected by dietary TRP, in both 30 (Table 2) and 60 d (Table 3) tests.

Mortality of fish fed different diets is presented in Table 4 and figures 1 and 2. Generally, fish fed with 0.25 TRP had lower mortality during Cu exposure, compared to other treatments, after both feeding trials.

After 30 d feeding, fish fed with 0.25% TRP had significantly lower mortality compared to the other treatments, however, there was no significant difference between the control group and fish fed on the diet supplemented with 0.5% TRP (Fig. 1).

Table 3: Two-way ANOVA for survival of the Caspian roach exposed to water copper and fed with tryptophan- and betaine-supplemented diet, over 60 d.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	7496.2	8	937.07	4.68	0.003
Intercept	23703.7	1	23703.7	118.5	< 0.0001
TRP	6607.4	2	3303.7	16.51	< 0.0001
BET	474.0	2	237.0	1.18	0.32
TRP × BET	414.8	4	103.70	0.51	0.72
Error	3600	18	200		
Total	34800	27			
Corrected Total	11096.3	26			

R Squared = 0.676 (Adjusted R Squared = 0.531)

Table 4. Mortality (%) of the Caspian roach exposed to water Cu and fed with tryptophan- and betaine-supplemented diet, over 30 and 60 d. Different letters show significant difference among the treatments, after 30 and 60 d, separately.

	30 d				60 d		
TRP	BET	Mean	Pooled S.E.	TRP	BET	Mean	Pooled S.E.
0	0	63.3 c	6.18	0	0	46.6 cd	7.93
	0.5	50.0 bc	6.18		0.5	56.6 d	7.93
	1	46.6 bc	6.18		1	36.6 bcd	7.93
0.25	0	3.33 a	6.18	0.25	0	6.66 a	7.93
	0.5	16.6 a	6.18		0.5	10.0 ab	7.93
	1	13.3 a	6.18		1	10.0 ab	7.93
0.5	0	63.3 c	6.18	0.5	0	23.3 bc	7.93
	0.5	43.3 bc	6.18		0.5	40.0 cd	7.93
	1	40.0 b	6.18		1	33.3 bcd	7.93

After 60 d feeding, fish fed with the 0.25% TRP diet had significantly lower mortality than the control fish and those fed with the diet containing 0.5% TRP treatments (Fig. 2). Also, fish in 0.5% TRP group had significantly lower mortality compared to the control group (Fig. 2).

Discussion

The present study showed that the Caspian roach is susceptible to Cu intoxication. Considering the Cu contamination of southern coast of the Caspian Sea, using dietary TRP can be a useful way to increase Caspian roach survival during stock rehabilitation. The reported level of Cu in the southern coast of the Caspian Sea and its tributaries is near 10% of LC50-96 h for the Caspian roach (De Mora et al., 2004; Khakpour et al., 2009; Hoseini and Jafar Nodeh, 2012), however, it could cause chronic toxicity and threatens fish health (Hoseini and Jafar Nodeh, 2012).

The present study showed that dietary TRP could increase tolerance to Cu intoxication in Caspian roach. The result is in line with the previous study on common carp (Hoseini and Hoseini, 2012). There are several potential reasons explaining how TRP enhanced the tolerance to Cu intoxication. It was reported that Cu exposure is stressful to fish (Flik et al., 2002; Firat et al., 2011; Hoseini and Hosseini, 2012). TRP have been reported to mitigate stress response in several fish species, via serotonergic system activation (Aldegunde et al., 1998, 2000; Winberg et al., 2001; Lepage et al., 2002; Hseu et al., 2003; Hoseini and Hosseini, 2010; Hoseini and Hosseini, 2012). Thus, one of the potential reasons for higher tolerance to Cu intoxication in TRP-fed groups might be mitigation of stress induced by Cu. On the other hand, De Boeck et al. (1995) reported that Cu exposure decreased significantly the brain serotonin level and serotonergic system disruption. TRP may counteract this phenomenon to increase fish survival during Cu exposure, as Winberg et al. (2001), Lepage et al. (2002) and Hseu et al. (2003) found an increase in brain serotonin content in TRP-fed fish. Likewise, Cu causes oxidative stress in fish (Roméo et al., 2000), thus, some protective effect of TRP during Cu toxicity might be related to its antioxidant properties, as previously reported by other authors (Christen et al., 1990; Weiss et al., 2002; Keithahn and Lerchl, 2005; Reyes-Gonzales et al., 2009).

In the present study, increase of dietary TRP from 0.25 to 0.5% influenced the fish tolerance to Cu toxicity adversely, which may be related to dietary amino acid imbalance and nutritional stress. Previous studies showed that high dietary TRP caused poor growth and physiological condition in other fish species (Papoutsoglou and Koustas, 2005; Papoutsoglou et al., 2005). Dietary requirement for TRP in Caspian roach is unknown which make it difficult to discuss about amino acid imbalance in

fish fed with 0.5% TRP.

In the present study, BET had no benefit for fish exposed to Cu. To our knowledge, there is no study in this topic to be compared with the present results. Kumar et al. (2012) found benefit of BET in endosulfan-exposed *L. ruhita*. The author suggested that BET could suppress stress and energy expenditure in endosulfan-exposed fish. Difference in fish species and experimental condition could potentially explain such contradictories between the two studies.

In conclusion, dietary TRP, especially 0.25%, can elevate tolerance to Cu intoxication in Caspian roach. TRP supplementation may have benefit in stock rehabilitation program of the Caspian roach, where fish may face Cu in the sea, rivers and rearing ponds.

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