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The protective effects of dietary garlic on common carp (*Cyprinus carpio*) exposed to ambient ammonia toxicity

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ABSTRACT

The present research aimed to investigate the potential effects of dietary garlic supplementation on the health status of common carp (*Cyprinus carpio*) exposed to ambient ammonia toxicity. The fish were fed with either of 0 (control), 0.5, 1 and 1.5% garlic levels for 35 days. They were then challenged with 0.5 mg/L ambient unionized ammonia-nitrogen for 3 h. Blood samples were taken before and after ammonia exposure to measure the antioxidant, enzymatic and immune responses of common carp. The results showed that garlic administration significantly decreased plasma glucose, cortisol and malondialdehyde (MDA) levels, and alanine transaminase (ALT), alkaline phosphatase (ALP), aspartate transaminase (AST) and glutathione peroxidase (GPX) activities. Also, it significantly increased plasma catalase (CAT), lysozyme, alternative complement (ACH50) and bactericidal activities, and immunoglobulin level, but had no effect on plasma superoxide dismutase activity. Ammonia exposure led to significant increases in plasma cortisol, glucose, MDA, SOD, CAT, GPX, ALT, AST, and ALP and decreases in ACH50, total Ig, bactericidal activity. Garlic supplementation significantly mitigated stress, oxidative stress and changes in plasma enzymatic activities in the fish exposed to ammonia. In conclusion, the current results suggest that dietary administration of garlic, especially at 1 and 1.5%, has beneficial effects to improve plasma antioxidant and immunological parameters and it was effective to mitigate the adverse effects of ammonia toxicity on antioxidant, enzymatic and immune responses in common carp.

1. Introduction

Common carp (*Cyprinus carpio*) is one of the most important farmed fishes in many countries.

The production of this species through aquaculture accounts for approximately 97.3% of its worldwide production (FAO, 2019). Recently, the intensive culture of common carp as a promising approach to meet demands has been developed in different countries (Hoseinifar et al., 2019). However, high stocking density may lead to accumulation of organic matter and toxic inorganic nitrogen as ammonia is the major part of the nitrogenous wastes of fish excrete (Adineh et al., 2019). In addition, a un-eaten feed can be decomposed in water and results in the formation of ammonia (Hoseini et al., 2019). Therefore, ammonia may reach an unsafe level and threaten fish health. Among nitrogenous compounds, ammonia is the most toxic for aquatic animals and high water ammonia causes to internal ammonia excretion impairment, which leads to an increase of ammonia uptake and even death (Kim et al., 2019). Previous researches showed the lethal effects of ammonia exposure on different fish species including common carp (Diricx et al., 2013), rainbow trout, *Oncorhynchus mykiss* (Wicks et al., 2002), big head carp, *Hypophthalmythys nobilis* (Sun et al., 2012), grass carp, *Ctenopharynodon idellus* (Xing et al., 2016), crucian carp, *Carassius auratus* (Ren et al., 2016) and yellow catfish, *Pelteobagrus fulvidraco* (Li et al., 2016). Moreover, it has been demonstrated that sub-lethal ammonia exposure leads to changes in feeding behaviour, reduced growth, physiological disturbances in blood chemistry, immune suppression and oxidative stress in farmed fishes (Hoseini et al., 2019; Kim et al., 2019; Zhang et al., 2018, 2019). Such changes, not only weaken fish against

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stressors and diseases, but also cause flesh quality deterioration (Zhang et al., 2016). Thus, preventing the harmful effects of ammonia on aquatic animals has a great impact on sustainable aquaculture.

In this regard, nutritional manipulation and use of dietary antioxidant additives due to their widespread beneficial effects on fish health during exposure to environmental ammonia have been considered by researchers. For example, exogenous taurine could mitigate the adverse effect of ammonia on the yellow catfish hematologic, antioxidant and immune responses (Zhang et al., 2018). In another study, Harsij et al. (2020) showed that the fish fed diets supplemented with antioxidants composing nano-selenium, vitamin C and E, and reared under sub-lethal ammonia exposure, significantly had better growth performances, and immune and antioxidant responses than those of fish in the control groups. In addition, previous studies have shown the antioxidant effects of medicinal plants and their extracts in the face of toxicants including ambient ammonia in various fish species (Abdel-Tawwab et al., 2015, 2017a, 2017b, 2018; Hamed and Abdel-Tawwab, 2017; Hoseini et al., 2018a, 2018b; Mahfouz, 2015; Taheri Mirghaed et al., 2019; Abdel-Tawwab et al., 2019). For example, Hoseini et al. (2019) observed that myrcene- or menthol-supplemented diets inhibited ammonia-induced tissue damage, anaemia and oxidative stress in common carp.

Garlic *Allium sativum* is an edible plant with medicinal characteristics and widely used in all over the world. It has antimicrobial, anticancer, antioxidant, hepatoprotective, and immunostimulant activity (Awad and Awaad, 2017). It has been demonstrated that garlic can improve growth performance and survival (Etyemez Büyükdeveci et al., 2018; Talpur and Ikhwanuddin, 2012), antioxidant activity.

(Metwally and Metwally, 2009; Mohebbi et al., 2012) and immune status (Fall and Ndong, 2011; Ghehdarijani et al., 2016) in different fish species. But, to the best of our knowledge, there is no information about the effects of garlic supplementation in fish exposed to ambient ammonia. Therefore, the present study was performed to evaluate the potential effects of garlic oral administration on some immunological and antioxidant parameters of common carp exposed to sublethal concentration of ambient ammonia.

2. Materials and methods

2.1. Preparation of garlic (G) powder and experimental diets

The fresh garlic was purchased from the local market, transferred to the laboratory and crushed after washing with deionized water. Then it was dried in an oven at 45 °C for 48 h and the homogenized powder was prepared using a mill. The powder was stored in zip pack at 4 °C until use. To prepare the experimental diets, 0.5% (0.5G), 1% (1G) and 1.5% (1.5G) garlic powders were added to the control (CTRL) diet (Table 1). To prepare the diets, feedstuffs were mixed well and 0.3 L water was added to each kg of the moisture. The dough was turned to sticks by a meat grinder. The sticks were crushed to form pellet (Hoseini et al., 2017).

2.2. Fish and experimental conditions

Ethics for procedures and animal use during the study were followed as described by Naderi et al. (2012). Fish (75 ± 7.36 g) were purchased from local farms and transported to the laboratory. During the one-week acclimatization fish were fed with the control diet. Then, a total number of 180 carp were stocked in 12 fiberglass tanks (100 L water volume) assigned to four treatments with three replicates (15 fish per tank). Fish were fed with either of the CTRL, 0.5, 1.0 and 1.5% G for 35 days. Feeding was done twice a day at a rate of 3% body weight (Ashouri et al., 2015). During experiment biometry was performed (three times) to adjust feed amount. Each tank was continuously aerated, siphoned and 70% of its water was replaced daily with clean water. Water temperature (21.3 ± 0.95 °C), pH (7.85 ± 0.56), dissolved oxygen (6.11 ± 0.67 mg/L) and total ammonia (0.22 ± 0.06 mg/L) were monitored every other day by Hach multiparameter meter HQ40d (Loveland, Colorado, USA) and Wagtech digital photometer 7100 (Berkshire, UK).

Table 1
Dietary formulation and proximate composition analysis of experimental diets containing different levels of garlic (Rajabiesterabadi et al., 2019).

Ingredient (g/kg)	CTRL	0.5G	1G	1.5G
Soybean meal[a]	16.5	16.5	16.5	16.5
Fish meal[b]	17.0	17.0	17.0	17.0
Poultry meal[c]	14.0	14.0	14.0	14.0
Wheat meal	38.1	37.6	37.1	36.6
Wheat gluten[d]	10.0	10.0	10.0	10.0
Fish oil	1.0	1.0	1.0	1.0
Soybean oil	1.0	1.0	1.0	1.0
Phytase[e]	0.5	0.5	0.5	0.5
Lysine[f]	0.6	0.6	0.6	0.6
Methionine[f]	0.3	0.3	0.3	0.3
Mineral mix[g]	0.5	0.5	0.5	0.5
Vitamin mix[h]	0.5	0.5	0.5	0.5
Garlic	0.0	0.5	1.0	1.5
Proximate composition				
Dry matter	91.0	91.1	90.4	90.2
Protein	40.3	40.2	39.9	39.8
Lipid	8.97	8.89	8.88	8.90
Ash	6.41	6.52	6.58	6.60

[a] Soyabean Co., Gorgan, Iran (crude protein 45.5%)
[b] Peygir co., Gorgan, Iran (crude protein 55.8%)
[c] Peygir co., Gorgan, Iran (crude protein 50.0%)
[d] Shahdineh Aran co., Isfahan, Iran (crude protein 78.3%)
[e] CheilJedang co., Seul, Korea
[f] Golbid co., Tehran, Iran (10,000 IU)
[g] The premix provided following amounts per kg of diet: Mg: 350 mg; Fe: 13 mg; Co: 2.5 mg; Cu: 3 mg; Zn: 60 mg; NaCl: 3 g; dicalcium phosphate: 10 g.
[h] The premix provided following amounts per kg of feed: A: 1000 IU; D3: 5000 IU; E: 20 mg; B5: 100 mg; B2: 20 mg; B6: 20 mg; B1: 20 mg; H: 1 mg; B9: 6 mg; B12: 1 mg; B4: 600 mg; C: 50 mg.

At the end of the feeding trial, the fish were challenged with 0.5 mg/L ambient unionized ammonia nitrogen for 3 h (Taheri Mirghaed et al., 2019). To achieve this amount, 3.01 g ammonium chloride were added to each experimental tank (100 L water volume), based on water temperature and pH.

2.3. Blood sampling and analysis

At the end of the 35-day feeding period, blood samples were taken before (AM-) and after a 3-h ammonia exposure (AM+). At each sampling time, two fish were sampled randomly from each tank and anesthetized in 100 mg/L eugenol solution. Then, using the heparinized syringes (5 mg heparin/ml blood), blood samples were taken from caudal vein and discharged into 2-ml plastic tubes. Samples were centrifuged at 1200 $\times g$ for 10 min and obtained plasma were stored at −70 °C until analysis. Each plasma sample was analyzed separately ($n = 6$).

Plasma glucose level was determined spectrophotometerically using Pars Azmun kits (Tehran, Iran) (Simakani et al., 2018). ELISA method (using IBL kit, Gesellschaft für Immunchemieund Immunbiologie, Germany) was applied to measure plasma cortisol level (Mazandarani et al., 2017). The inter- and intra-assay variation were 8.96 and 10.4%, respectively.

Plasma superoxide dismutase (SOD) level was estimated by measuring the rate of cytochrome C reduction and using a commercial kit (ZellBio, GmbH, Veltinerweg, Germany) (McCord and Fridovich, 1969). Plasma catalase (CAT) level was measured following Góth (1991) method by measuring the rate of hydrogen peroxide decomposition. Plasma glutathione peroxidase (GPx) level was measured using a commercial kit (ZellBio, GmbH, Veltinerweg, Germany) by estimating the glutathione oxidation rate. Plasma malondialdehyde (MDA) level was determined by the thiobarbituric acid method and using a commercial kit (ZellBio, GmbH, Veltinerweg, Germany) (Yousefi et al., 2018).

Plasma lysozyme was evaluated by turbidimetric method using *Micrococcus luteus* as the target in phosphate buffer (pH = 6.2) as described by Ellis (1990). Plasma alternative complement haemolytic (ACH50) level was measured based on hemolytic activity with gelatin-veronal buffer containing EGTA and Mg^{2+} (pH = 7) as the medium and sheep white blood cell (RBC) as the target (Yano, 1992). According to Siwicki and Anderson (1993), total immunoglobulin (Ig) was measured after polyethylene glycol precipitation of Ig and subtraction of initial and final total protein.

Plasma bactericidal activities were evaluated against *Aeromonas hydrophila*, as previously described by Zargari et al. (2018). Briefly, the bacterium suspended at 546 nm and 0.5 optical density. Dilution of suspension was performed 5 times (1:10) and the least dilution was used for this assay. 1 mL suspension was added to 100 μL plasma and incubated at 20 °C for 1 h. Then, plasma and bacteria mixture were cultured on nutrient agar plates (with three replicates). The number of colonies was counted after 24 h incubation at 25 °C. Phosphate buffered saline was used instead of serum in the control samples. The colony numbers were converted to bactericidal activity using the following formula:

Bactericidal activity = 100, 000 × (1/colony number)

Alanine transaminase (ALT), alkaline phosphatase (ALP) and aspartate transaminase (AST) activities were evaluated with spectrophotometric method using commercial kits (Pars Azmun kits, Tehran Iran), as previously reported in common carp (Hoseini et al., 2012, 2018).

2.4. Statistical analyses

Data were tested for normal distribution using the Shapiro-Wilk test; accordingly, all data except GPx, total Ig and AST were log-transformed before ANOVA. The data were analyzed by two-way ANOVA (garlic levels × ammonia exposure). When there was an interaction effect of garlic levels × ammonia exposure (all data except total Ig and AST), one-way ANOVA was used for the data analysis. In this case, the data normality (Shapiro-Wilk test) and variance homogeneity (Levene's test) were tested first, and log-transformation was applied when necessary (bactericidal activity). All analyses were conducted in SPSS v.21.

3. Results

Plasma cortisol and glucose levels of fishes significantly elevated after exposure to ambient ammonia (Fig. 1). All garlic treatments markedly decreased the plasma cortisol and glucose levels compared to the control group (n = 12; $P < .001$). A significant interaction was shown between garlic levels and ammonia exposure for these parameters. Ammonia exposure led to a significant increase in cortisol and glucose levels, whereas the highest levels were recorded in the control group.

Plasma SOD and CAT activity of fishes significantly elevated after exposure to ambient ammonia (Fig. 2). Garlic levels had no significant effects on SOD; whereas significantly increased CAT activity ($P < .001$). Dietary garlic levels and ammonia exposure had interaction effects on plasma SOD and CAT activities. Ammonia exposure led to a significant increase in SOD activity and the highest increase was related to the control group. Also, ammonia exposure significantly increased CAT activity in the control and 0.5% G groups; but had no effects on 1 and 1.5% G groups.

Plasma GPX activity and MDA level of fishes markedly increased after exposure to ambient ammonia (Fig. 3). Dietary garlic supplementation significantly decreased plasma GPX activity ($P < .006$) and

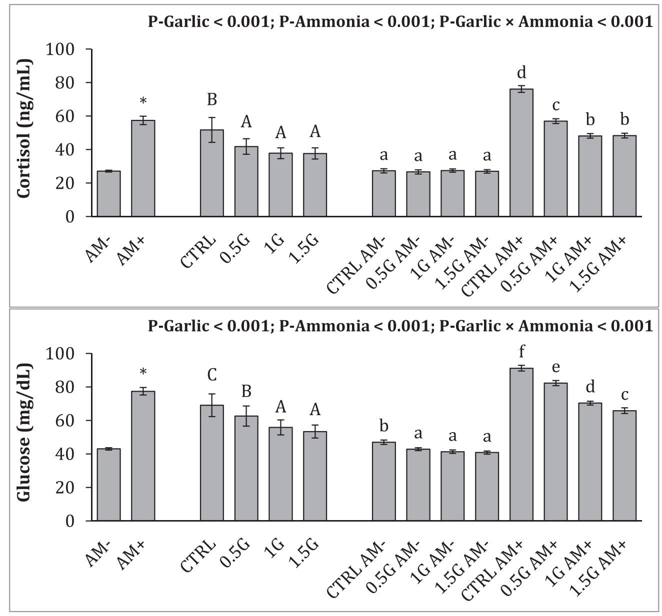

Fig. 1. Effects of dietary garlic administration and ammonia exposure on cortisol and glucose levels (mean ± SE) in the blood plasma of common carp. Asterisk show significant effects of ammonia exposure (n = 24). Uppercase letters show significant effects of dietary garlic levels (n = 12). Lowercase letters show significant interaction effects of garlic levels and ammonia exposure (n = 6).

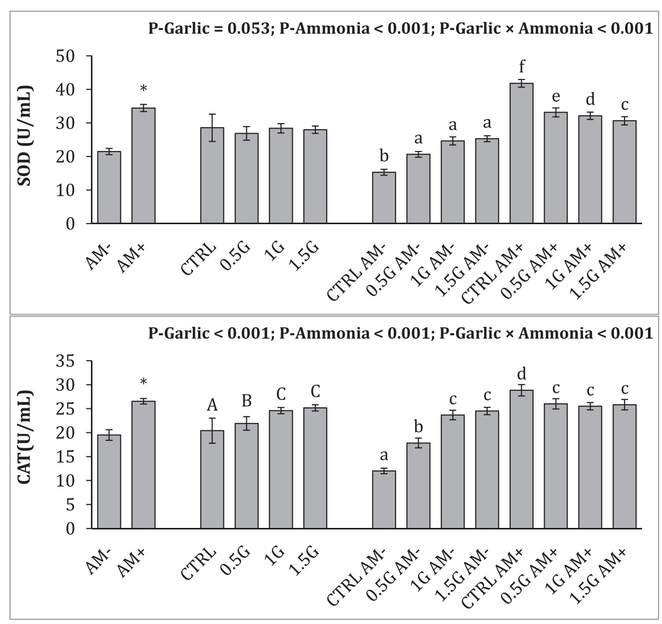

Fig. 2. Effects of dietary garlic administration and ammonia exposure on SOD and CAT activity (mean ± SE) in the blood plasma of common carp. Asterisk show significant effects of ammonia exposure (n = 24). Uppercase letters show significant effects of dietary garlic levels (n = 12). Lowercase letters show significant interaction effects of garlic levels and ammonia exposure (n = 6).

MDA level in all treatments compared to the control group ($P < .001$). There was an interaction effect between ammonia exposure and dietary garlic levels on plasma GPX activity and MDA level. Ammonia exposure led to an increase in plasma GPX activity of control and 0.5% G groups but had no effects on 1 and 1.5% G groups. Ammonia exposure markedly increased plasma MDA levels, whereas the highest increase was observed in the control compared to other groups.

According to the results shown in Fig. 4, plasma lysozyme activity of

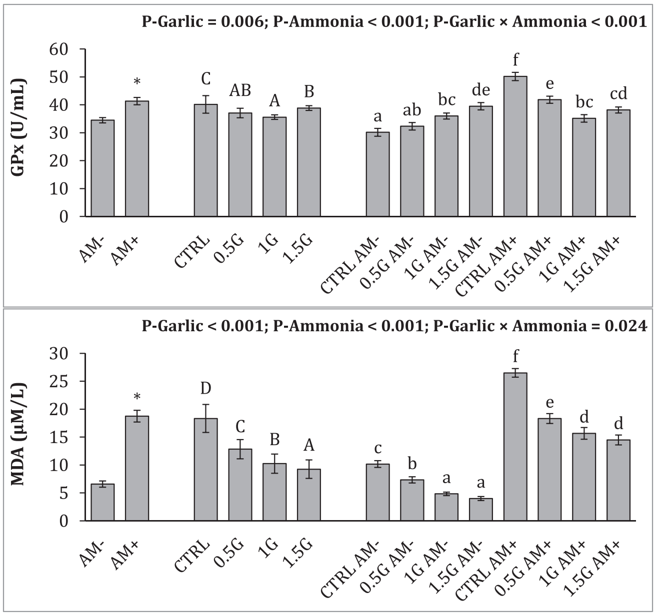

Fig. 3. Effects of dietary garlic administration and ammonia exposure on GPX activity and MDA level (mean ± SE) in the blood plasma of common carp. Asterisk show significant effects of ammonia exposure (n = 24). Uppercase letters show significant effects of dietary garlic levels (n = 12). Lowercase letters show significant interaction effects of garlic levels and ammonia exposure (n = 6).

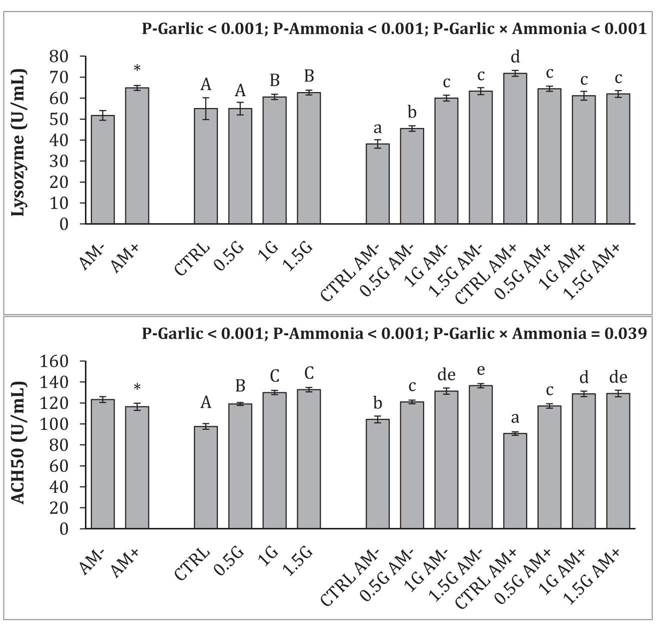

Fig. 4. Effects of dietary garlic administration and ammonia exposure on lysozyme and alternative complement activities (mean ± SE) in the blood plasma of common carp. Asterisk show significant effects of ammonia exposure (n = 24). Uppercase letters show significant effects of dietary garlic levels (n = 12). Lowercase letters show significant interaction effects of garlic levels and ammonia exposure (n = 6).

fishes significantly increased after exposure to ambient ammonia, whereas ACH50 activity decreased (P < .001). Dietary garlic supplementation significantly increased plasma lysozyme activity (P < .001) in 1 and 1.5% G groups compared to the control and 0.5% G groups (P < .001). Also, dietary garlic levels increased the ACH50 activity compared to the control group (P < .001). Dietary garlic levels and ammonia exposure had interaction effects on plasma lysozyme and

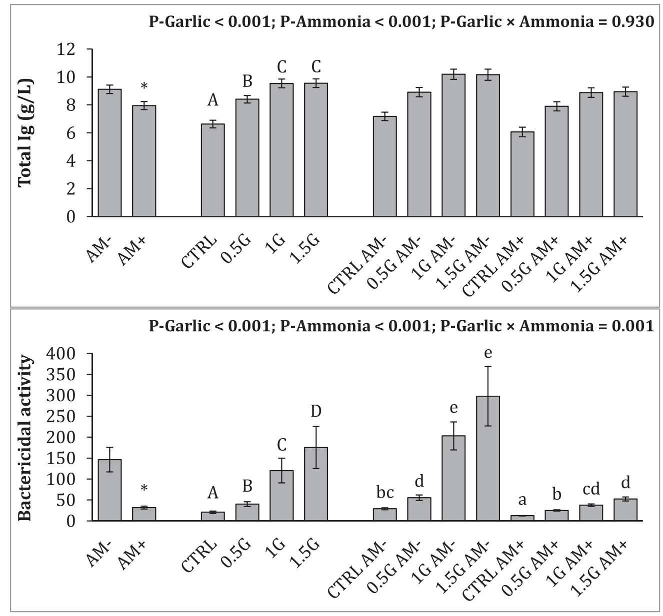

Fig. 5. Effects of dietary garlic administration and ammonia exposure on immunoglobulin levels and bactericidal activities (mean ± SE) in the blood plasma of common carp. Asterisk show significant effects of ammonia exposure (n = 24). Uppercase letters show significant effects of dietary garlic levels (n = 12). Lowercase letters show significant interaction effects of garlic levels and ammonia exposure (n = 6).

ACH50 activities. Ammonia exposure led to an increase in plasma lysozyme activity of the control and 0.5% G groups; but had no effects on 1 and 1.5% G groups. After ammonia exposure, ACH50 activity significantly decreased in the control group; but no change was observed in other treatments.

Plasma total Ig level and bactericidal activities significantly (P < .001) decreased in all treatments after ammonia exposure (Fig. 5). Dietary garlic administration in all levels significantly increased plasma Ig level and bactericidal activity compared to the control group (P < .001). Dietary garlic levels and ammonia exposure had no interaction effects on plasma Ig level; but their interaction effects observed on plasma bactericidal activity. Ammonia exposure led to a significant decrease in plasma bactericidal activity and the highest decrease was related to the control group compared to other groups (P < .001).

Ammonia exposure led to a significant increase in plasma ALT, AST and ALP activities (Fig. 6). Dietary garlic supplementation significantly decreased plasma ALT, AST and ALP activities in all treatments compared to the control group (P < .001). There were any significant differences between 1 and 1.5% G groups in the case of ALT and AST activities, whereas the lowest level of ALP was observed in the 1.5% G group. Dietary garlic levels and ammonia exposure had interaction effects on plasma ALT and ALP activities but had no interaction effects on plasma AST activities. Ammonia exposure markedly increased plasma ALT and AST activities in all treatments, whereas the highest increase was observed in the control compared to other groups.

4. Discussion

Ammonia poisoning is a very serious threat to aquatic organisms, as most of the bony fish such as common carp *C. carpio* (Hoseini et al., 2019), grass carp *C. idellus* (Xing et al., 2016), crucian carp *C. auratus* (Ren et al., 2016) and rainbow trout *O. mykiss* (Harsij et al., 2020) are sensitive to its toxicity. The previous studies have proved that ammonia toxicity cause growth degradation and health deterioration (Peyghan and Takamy, 2002; Xing et al., 2016) oxidative stress (Zhang et al.,

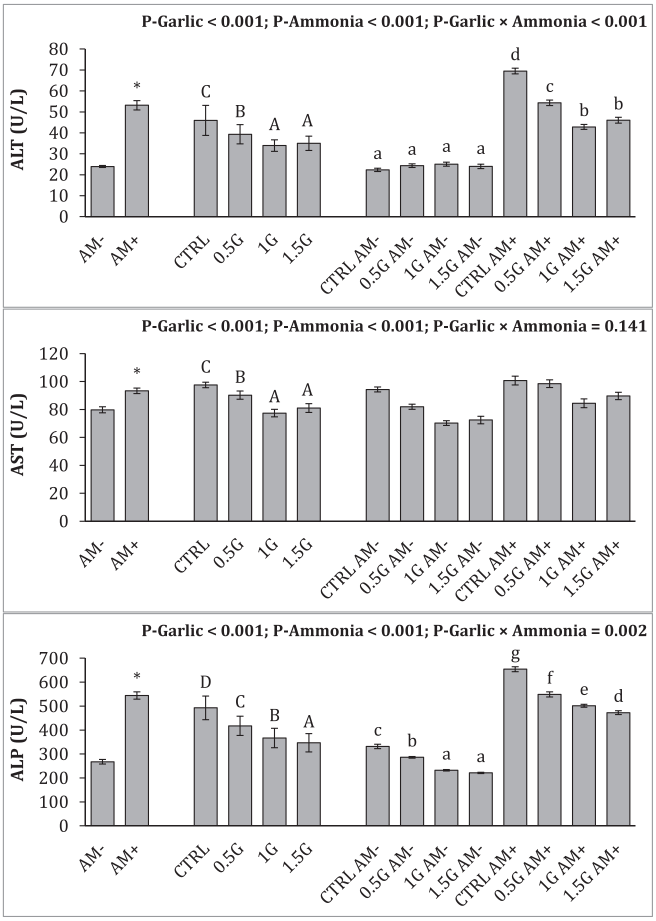

Fig. 6. Effects of dietary garlic administration and ammonia exposure on ALT, AST and ALP activities (mean ± SE) in the blood plasma of common carp. Asterisk show significant effects of ammonia exposure (n = 24). Uppercase letters show significant effects of dietary garlic levels (n = 12). Lowercase letters show significant interaction effects of garlic levels and ammonia exposure (n = 6).

2018) and tissue damages (Hoseini et al., 2019), leading to suppression of stress and disease resistance in fish (Ackerman et al., 2006). Nutritional supplements including medicinal herbs with antioxidant properties can be used to counter these threats (Awad and Awaad, 2017; Rajabiesterabadi et al., 2020). Garlic includes two major groups of antioxidant components, flavonoids and sulfur-containing compounds (diallyl disulfide and s-allyl cysteine) (Sharma et al., 2010). Flavonoids (as natural antioxidants) are oxidized by free radicals, resulting in a more stable, less-reactive radical (Nijveldt et al., 2001). Diallyl disulfide removes hydroxyl radicals, and s-allyl cysteine suppresses the formation of superoxide (Chung, 2006). Although garlic and its extracts have been widely used in aquaculture research, no studies have been reported on the potential effects of garlic against toxicity with ambient ammonia.

Fish may react to environmental stress by elevation of plasma cortisol and glucose as the primary and secondary responses (Barton, 2002). In line with the present results, the previous studies demonstrated that ambient ammonia elevation leads to an increase in plasma cortisol and glucose levels (Knoph and Olsen, 1994; Randall and Tsui, 2002); such changes provide the energy needed to deal with environmental stress (Taheri Mirghaed et al., 2018). Therefore, the present results confirm that garlic can successfully counteract the negative effects of ammonia toxicity through suppression of increased cortisol and glucose. Similarly, previous studies showed that anthraquinone extract and cineole prevented the increase in these factors in fish exposed to crowding stress (Taheri Mirghaed et al., 2019; Xie et al., 2008).

In the present study, after acute ammonia intoxication, the activity of antioxidant enzymes including SOD, CAT and GPX increased, suggesting the activation of antioxidant system to counteract oxidative conditions under the ammonia toxicity (Zhang et al., 2018). The highest activity of the antioxidant enzymes were observed in the control group; whereas, the garlic-supplemented groups showed suppressed increase in the enzymes' activity. It can hold the opinion that the lowest antioxidant capacity was related to the control group. In fact, total antioxidant capacity means the ability of antioxidants to counter and eliminate harmful free radicals in the blood and cells (Martínez-Álvarez et al., 2005). The present results showed that antioxidant enzyme activities had a positive and negative correlation with total antioxidant capacity in fish fed with garlic and control diets, respectively. Thus, raising the antioxidant enzyme activity in the control group without the increased antioxidant capacity may due to a "toxic excitement effect" (Zhang et al., 2018). Also, the present study showed that dietary garlic supplementation can mitigate the increment of MDA production compared with the control group. It can be explained by the increased antioxidant capacity in fish fed garlic diets, as above mentioned. It is reported that garlic extract can promote antioxidant activity by scavenging reactive oxygen species, boosting the cellular antioxidant enzymes, glutathione peroxidase and enhancing glutathione in the cells (Borek, 2001). The previous study showed that dietary garlic administration improved the antioxidant activity in Nile tilapia (*Oreochromis niloticus*) (Metwally and Metwally, 2009). Similarly, Hoseini et al. (2019) founded that dietary supplementation of myrcene- or menthol enhanced antioxidant activity and alleviated increased level of MDA in common carp exposed to ambient ammonia.

Fish immunity is suppressed by toxicants exposure (Abdel-Tawwab et al., 2019; Wang et al., 2015; Zhang et al., 2018). Lysozyme, a lytic enzyme is one of the non-specific humoral molecules in fish. This enzyme can act in opsonization of target cells and activate the plasma molecules (the complement system) involved in the control of inflammation (Lee, 2015). It was noticed that 1 and 1.5% garlic supplementations significantly improve the lysosome level before ammonia exposure. Moreover, these garlic levels could mitigate the ammonia-induced lysozyme elevation. In line with our results, Taheri Mirghaed et al. (2019) showed that Dietary 1,8-cineole significantly prevented ammonia-induced lysozyme elevation in common carp.

ACH50 activity and total Ig levels as indicators of immune status may be suppressed in fish exposed to toxicants (Sharifian et al., 2015; Wang et al., 2014). Dietary garlic levels significantly enhanced these factors in plasma compared to the control group, suggesting immune stimulation by garlic supplementation. Also, 1 and 1.5% garlic levels mitigate the decreasing of ACH50 activity after exposing to ambient ammonia. Similar results were observed in *C. carpio* (Hoseinifar et al., 2018) and *O. mossambicus* (Wu et al., 2010) fed with dietary *Eriobotrya japonica* and *Toona sinensis* extracts, respectively. Taheri Mirghaed et al. (2019) reported that plasma ACH50 activities and total Ig levels decreased after exposure to ammonia, but 0.5% dietary 1,8-cineole could mitigate the suppression of these factors compared to the control group.

It has been demonstrated that garlic takes effect as an immunostimulant by boosting bactericidal activities (Awad and Awaad, 2017; Erguig et al., 2015). In the present study, garlic levels significantly increased the plasma bactericidal activity and mitigate its decrement after exposure to ambient ammonia. The results are in line with the previous studies in Asian seabass, *Lates calcarifer* (Talpur and Ikhwanuddin, 2012) and rainbow trout (Nya and Austin, 2009).

ALP, AST, and ALT are non-functional enzymes which mainly found in the liver and kidney (Ghelichpour et al., 2020). Increased circulating levels of these enzymes are a sign of tissue (De Smet and Blust, 2001; Taheri Mirghaed et al., 2019). According to the present results, ammonia exposure significantly increased plasma ALT, AST and ALP levels of common carp. It may be due to the vital tissues damage or hemolysis caused by ammonia exposure (Taheri Mirghaed et al., 2017; Hoseini et al., 2018b). As impaired oxidative enzyme activities and elevated level of MDA can support this hypothesis. Previous studies have demonstrated that ammonia exposure caused tissue damage and increased these enzymes levels in the blood of different fish species (Hoseini et al., 2019; Peyghan and Takamy, 2002; Taheri Mirghaed et al., 2019). In the present study, garlic levels significantly decreased the plasma ALT, AST and ALP levels and mitigate their elevation after exposure to ambient ammonia compared to the control group. Masjedi et al. (2013) were observed the preventive effects of garlic on elevation of these enzymes in serum of the diabetic Rats. Similar results were reported in fish fed with 1,8-cinoele (Taheri Mirghaed et al., 2019) and myrcene- or menthol-supplemented diets (Hoseini et al., 2019) and exposed to ambient ammonia. Having antioxidant components (flavonoids and sulfur-containing components), garlic may enhance cell membrane stabilization and protect tissues against free radical-mediated toxic damages. Having antioxidant components (flavonoids and sulfur-containing components), garlic may enhance cell membrane stabilization and protect tissues against free radical-mediated toxic damages, that resulted in decreased ALT, AST and ALP levels.

In conclusion, the present results demonstrated that dietary garlic supplementation especially at 1 and 1.5% diets, successfully enhanced most of the measured parameters. Also, it mitigates the adverse effect of ammonia exposure characterized by improving antioxidant activities and decreasing MDA production. Further studies are encouraged to monitor flesh quality and taste following feeding the fish with garlic-supplemented diet and exposed to ammonia, as formation of MDA in fish flesh changes its quality and taste.

Author statement

The authors have participated equally in all parts of this study.

Declaration of Competing Interest

None.

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