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Assessment of vital organ histopathology and plasma oxidative conditions of rainbow trout *Oncorhynchus mykiss* reared in earthen saltwater pond

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Abstract. The aim of this study was to compare gill, kidney, liver and gut histopathology, and plasma antioxidant markers of rainbow trout *Oncorhynchus mykiss* reared in saltwater earthen ponds in Gomishan, Iran. To this, 10000 fish were distributed in a three-ha earthen pond and 150 fish in three fiberglass tanks (2000L). Blood samples were taken after 3 months rearing with same commercial feed. The source of fish and feed was similar between the saltwater pond and fiberglass tanks. After the 3-month rearing, gill, kidney, liver and gut samples were taken from the pond fish; whereas, blood samples were taken from both the pond and tank fish. There was no significant difference in water temperature, dissolved oxygen and pH between the pond and tanks; however, water salinity and ammonia was higher in the pond compared to the tanks. Plasma superoxide dismutase and glutathione peroxidase activity of the fish in earthen ponds were significantly higher than those fish reared in fiberglass tanks; however, there was no significant in thiobarbituric acid reactive substances between the pond and tanks. The fish had various histopathological symptoms including primary and lamella hyperplasia, lamellar fusion and epithelial lifting. In the kidney section, the fish showed glomerulus shrinkage and/or disappearance, melanomacrophage aggregates and hematopoietic tissue necrosis. These fish showed necrosis and melanomacrophage aggregates in liver and goblet cell hypertrophy in gut. The results suggest that the fish in the earthen pond faced stressful conditions, which might be due to water salinity and ammonia; however, other possible factors, such as pollutants and different feeding regimen must be considered.

Key words: trout, earthen pond, antioxidant system, saltwater, histopathology, Gomishan, Iran

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Introduction

Gomishan site of shrimp culture is important in economy of the Goletan province, Iran. The site area is near 4000 ha and had total production of 2000 tons of shrimp in 2018. Despite its importance, a problem of the site is seasonality of work period, because the water temperature is suitable for shrimp culture only four months a year. As a result, the employees hire workers seasonally, which is considered as a social crisis [1]. A solution for this problem is to introduce an aquatic species to be cultured in the second term of year (autumn and winter). In this case, rainbow trout (*Oncorhynchus mykiss*) seems to be fitted with the environmental conditions of the site (temperature and salinity); however, it is necessary to monitor the fish health in these earthen ponds [2].

Histopathological studies are reliable tools to assess fish health, illustrating the fish responses to toxicant exposure and water salinity [3]. In this case, fish gill is the major organ for hydromineral control, responding morphologically to water salinity [4]. Liver is the main site of metabolism and the target of toxicants, showing wide range of morphological and histological alteration in response to toxicants [5]. Fish kidney is involved in salinity adaptation, which responds morphologically to toxicants [5]. Fish gut is an important organ in immune defense, which responds to toxicant and dietary compounds [3]. Monitoring of these organs provides useful information about the fish overall health.

Oxidative stress is a condition in when pro-oxidant compounds exceed antioxidant defense [6, 7]. In this case, stressful conditions lead to oxidative stress. Superoxide dismutase (SOD) and glutathione peroxidase (GPx) are antioxidant enzymes collectively converting superoxide ions to water and oxygen [7, 8]. Therefore, they are important in monitoring oxidative conditions of fish. However, these enzymes show different either elevation or demotion under stressful conditions. As a result, thiobarbituric acid reactive substances (TBARS) are measured as a relevant marker of lipid peroxidation and oxidative stress [9].

According to above, the aim of the present study was to investigate rainbow trout health (histopathology and antioxidant responses) in earthen ponds of the Gomishan site.

Materials and methods

This study was conducted in an earthen pond in the Gomishan site of shrimp culture. The pond was filled with the Caspian Sea water in spring, and used for shrimp culture during spring and summer. Then the pond water was drained and the pond bottom was dried for 2 months. Thereafter, the pond was refilled with the sea water for trout culture. 10000 rainbow trout (~35 g) were stocked in a 3-ha pond. For comparison of blood antioxidant marker with a reference, 150 rainbow trout, with the same origin of the pond fish, were socked in three fiberglass tanks (2000 L). The fish in the pond and fiberglass tanks were reared for three months using a commercial feed (Faradaneh Co., Tehran, Iran; 40—44 % protein, 12—16 % fat). Feeding was performed twice a day based on 3 % of biomass. Water of the pond was static; whereas, water flow rate in the tanks was 0.3 L/min. kg fish. Water physiochemical parameters were measured in the pond and tanks monthly.

At the end of the three-month rearing, 12 fish were sampled from either the pond or tanks and immediately anesthetized with 100 mg/L eugenol. Immediately after the anesthesia, blood samples were taken from caudal vein using heparinized syringes and collected in 2-mL tubes. The blood samples were centrifuged for 10 min for plasma separation. The resultant plasma was kept at –20 for further analyses. After the blood sampling, gill, liver, kidney and gut samples were taken from the pond fish. The organs were fixed in 10 % buffered formalin for histopathological examination.

Plasma SOD, GPx and MDA were determined using commercial kits (ZellBio, GmbH, Veltinerweg, Germany), as previously reported M.A. Taheri et al. [10]. The fish organs were parafinized, sectioned (4μm thickness) and stained (hematoxylin-eosin) according to A. Hedayati et al. [11]. Histopathological damages were determined according to Haschek et al. [3] and R.J. Roberts [12].

Plasma and water physiochemical parameters were subjected to t-test. Data were presented as mean ± SD. All analyses were performed using SPSS v. 22.

Results and discussion

Water physiochemical parameters in pond and tanks are presented in Table 1. The pond water had significantly higher salinity and unionized ammonia compared to the tanks. Nevertheless, there was no significant difference in water temperature, dissolved oxygen and pH.

Table 1

Water physiochemical characteristics in the pond and tank. Different letters show significant difference between the pond and tank

Physiochemical characteristics	Pond	Tank
Temperature (ºC)	13 ± 1*a*	14 ± 1*a*
Dissolved oxygen (mg/L)	8.88 ± 0.55*a*	8.56 ± 0.84*a*
Salinity (g/L)	20.7 ± 1.12*b*	2.88 ± 0.12*a*
pH	8.65 ± 0.55*a*	7.52 ± 0.69*a*
Unionized ammonia (μg/L)	10.0 ± 1.0*b*	2.0 ± 0.40*a*

Plasma antioxidant parameters are presented in Table 2. The fish reared in the tanks had significantly lower SOD and GPx activities; but there was no significant difference in plasma TBARS levels between the fish.

Table 2

Plasma SOD, GPx and MDA in the fish reared in the pond and tanks. Different letters show significant difference between the pond and tank

	SOD (U/L)	GPx (U/L)	TBARS (μM/L)
Pond	35.1 ± 5.89*b*	45.5 ± 7.32*b*	6.85 ± 1.98*a*
Tank	23.7 ± 4.55*a*	30.0 ± 5.21*a*	5.21 ± 1.57*a*

Gill section of the fish reared in Gomishan pond is presented in Figure 1. The fish had various histopathological symptoms including primary and lamella hyperplasia, lamellar fusion and epithelial lifting. In the kidney section, the fish showed glomerulus shrinkage and/or disappearance, melanomacrophage aggregates and hematopoietic tissue necrosis (Figure 2). These fish showed necrosis and melanomacrophage aggregates in liver (Figure 3) and goblet cell hypertrophy in gut (Figure 4).

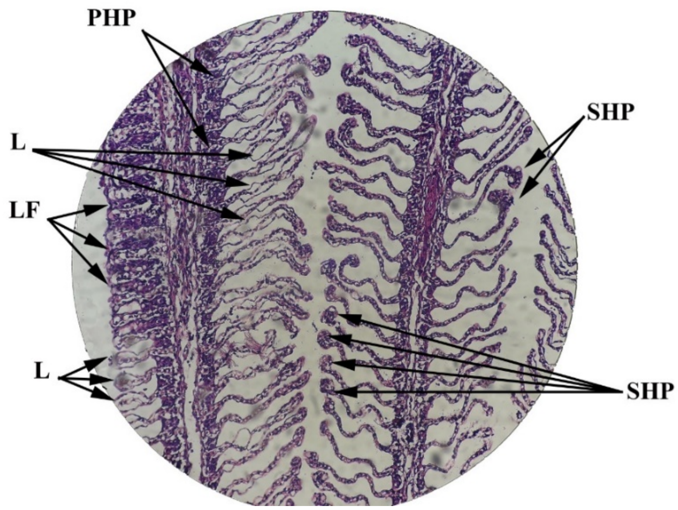

Fig. 1. Gill section of the fish reared in the pond. PHP: primary lamella hyperplasia; L: epithelial lifting; LF: lamellar fusion; SHP: secondary lamella hyperplasia. Hematoxylin-eosin staining (20×)

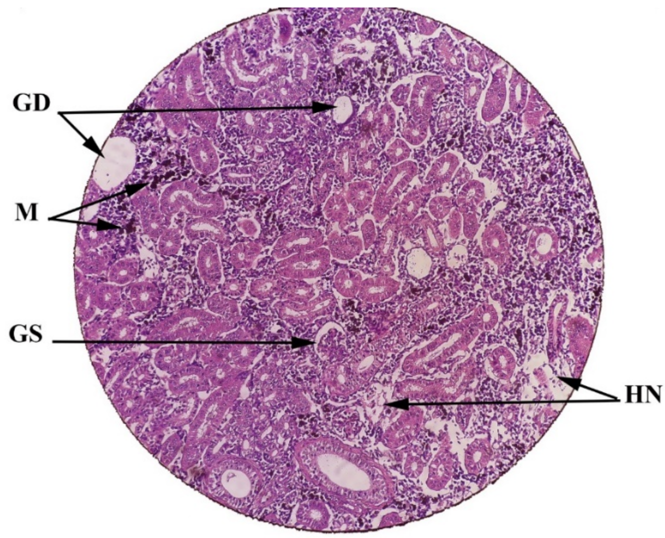

Fig. 2. Kidney section of the fish reared in the pond. GD: glomerulus disappearance; M: melanomacrophage aggregates; GS: glomerulus shrinkage; HN: hematopoietic tissue necrosis. Hematoxylin-eosin staining (20×)

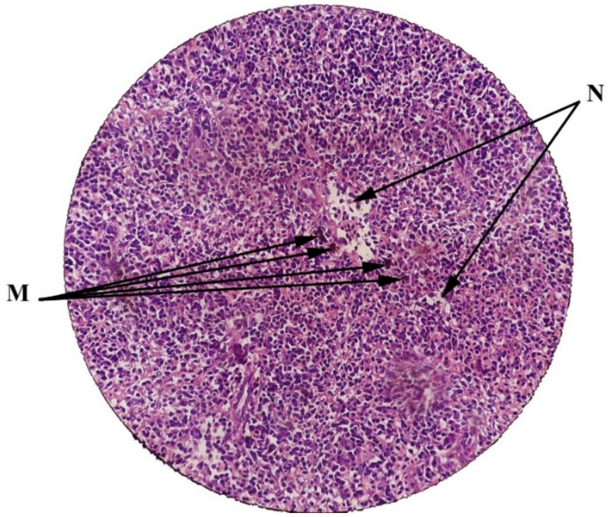

Fig. 3. Liver section of the fish reared in the pond. N: necrosis; M: melanomacrophage aggregates. Hematoxylin-eosin staining (20×)

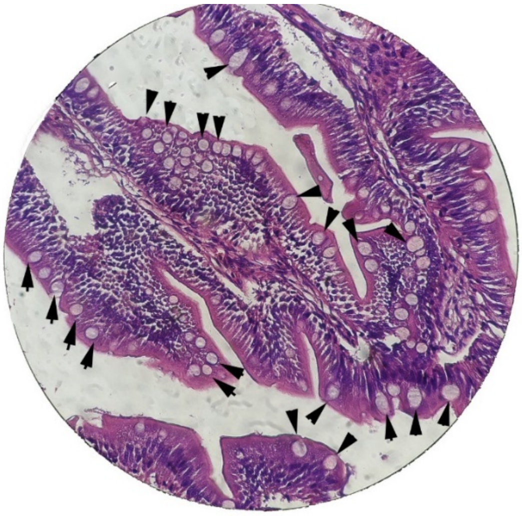

Fig. 4. Gut section of the fish reared in the pond. Arrow heads show hypertrophy of the goblet cells. Hematoxylin-eosin staining (40×)

The present study showed that saltwater earthen ponds of Gomishan site had different environment conditions compared to freshwater farms of rainbow trout culture. In these ponds, due to higher pH and static water [13—15], unionized ammonia is higher than the freshwater systems. Higher ammonia along with saltwater are stressful for rainbow trout, thus, affect the fish health [13—16].

The pond fish showed higher antioxidant enzymes' activity but did not elevated TBARS, compared to the tank fish. This suggests activation of antioxidant system, but not oxidative stress occurrence [17]. Several factors might be involved in antioxidant system activation. Ammonia was found to induce oxidative stress in fish leading to elevated TBARS [18]. Accordingly, oxidative conditions were not so severe as to cause oxidative stress in the pond fish, and activation of antioxidant system counteracted such mild oxidative condition. Beside ammonia, osmotic stress causes oxidative condition, triggering the fish antioxidant system [19, 20]. Therefore, higher water ammonia and salinity contributed to activation of antioxidant system of the pond fish. Moreover, considering the wide difference between the pond and tank media, other possibility such as pathogens, pollutants, etc. could be considered.

Gill is the main organ for hydromineral control in freshwater fish [4]. It has been reported that freshwater fish exposed to saltwater expressed some morphological changes in gill structure [21]. Hyperplasia is a defensive mechanism to thicken gill membrane for reducing exchange capacity between the internal and external media of the fish body. This reduces the entrance of harmful substances, including hyperosmotic water and ammonia, from water to the fish body [22]. Epithelial lifting provides distance between the fish internal body and ambient water to reduce harmful substances entrance to the fish body [22]. However, lamellar fusion occurs in adjacent secondary lamella and extensively decreases respiratory capacity [22].

Beside gill, kidney is involved in water and ions regulation in fish [21]. When freshwater fish enter hyperosmotic media, water is passively excreted from the fish body and the animal must actively drink along with suppressing glomerulus filtration. Accordingly, fish have small glomerulus in freshwater [23]. The fish reared in Gomishan ponds had both shrinked and disappeared glomerulus to adapt higher water salinity. However, melanomacrophage aggregates and necrosis in hematopoietic tissues suggest that there was other problem in these fish. The liver section showed similar symptoms, supporting such hypothesis. One of the reasons might be higher water ammonia, which causes damage in fish kidney [24]. On the other hand, other toxicants such as metals might contribute to these damages, as they are present in the Caspian Sea water [25].

The pond-reared fish showed increased number and size of goblet cells in gut. These cells are responsible for mucus secretion and increase in their number and size translated to higher mucus secretion [26]. The reason for these changes is not clear, however, the pond water has microbial community different from the tank water. It is possible some microbes stimulate the fish gut and lead to higher mucus secretion [27]. On the other hand, despite the tank fish that eat solely pellet feeds, the pond fish eat on both pellet feed and natural foods in pond (e.g. insects, worms, etc.). Thus, it is expected the pod fish have gut structure different from the tank fish.

In conclusion, the fish in the earthen pond faced stressful conditions, which might be due to water salinity and ammonia. It is necessary to apply managerial practice to suppress such problems and augment the fish health and welfare.

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Научная статья

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Оценка гистопатологии жизненно важных органов и условий окислительных процессов цитоплазмы радужной форели *Oncorhynchus mykiss*, выращенной в глиняном пруду с морской водой

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Аннотация. Цель данного исследования заключалась в сравнении гистопатологий жабр, почек, печени и кишечника, и оценке активности системы антиоксидантной защиты радужной форели (*Oncorhynchus mykiss*), выращенной в глиняных прудах с морской водой в г. Гомишан в Иране. Для этого в глиняный пруд площадью 3 га поместили 10000 особей и 150 рыб распределили в трех резервуарах из стекловолокна (2000 л). Форель выращивали в течение трех месяцев, после чего у рыб, культивируемых в пруду и резервуарах, были взяты образцы крови, в то время как образцы тканей жабр, почек, печени и кишечника были взяты для гистологического анализа только у прудовых рыб. Температура воды, содержание растворенного в воде кислорода и значение pH в пруду и резервуарах имели несущественные различия; однако такие параметры как соленость воды и содержание аммиака имели более высокие показатели в пруду. Активность цитоплазматической супероксиддисмутазы и глутатионпероксидазы у рыб, культивируемых в глиняных прудах, была значительно выше по сравнению с особями, выращенными в стекловолоконных резервуарах. Тем не менее, между прудом и резервуарами не было значительной разницы по количеству веществ, реагирующих с тиобарбитуровой кислотой. После проведения гистологического анализа у рыб были выявлены такие патоморфологические изменения как первичная гиперплазия ламелл, слияние ламелл и эпителиальный лифтинг. На поперечном сечении почек у рыб наблюдали сморщивание или исчезновение клубочков, скопления меланомакрофагов и некроз гемопоэтической ткани. Кроме того, у этих рыб были обнаружены следы некрозов и скопления меланомакрофагов в печени, а также гипертрофия бокаловидных клеток кишечника. Результаты исследований показывают, что в глиняном пруду рыба оказалась под воздействием стрессовых факторов, среди которых могут быть параметры солености воды и содержания аммиака. Но необходимо учитывать и другие возможные причины, например, загрязняющие вещества и различные режимы кормления.

Ключевые слова: форель, глиняный пруд, система антиоксидантной защиты, морская вода, гистопатология, Гомишан, Иран

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