Original Article

Assessment of Skin Pathological Responses in the Yellowfin Seabream *(Acanthopagrus latus)* under the *Aeromonas hydrophila* Exposure

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Received: 10.07.2017 *Accepted: 13.08.2017*

ABSTRACT

Background: Bacterial diseases in cultured fish are considered the main problem to aquaculture system. Skin is the structure that covers the body in fish. Skin histopatological alterations were used to assess the effects of *Aeromonas hydrophila* exposure on the yellowfin seabream (*Acanthopagrus latus*).

Methods: In this regard, 90 *A. latus* were exposed to sublethal concentrations of *A. hydrophila* (10^3,10^6 CFU/ml) for 3 weeks.

Results: Some more severe alternations found in the skin of fish exposed. The most frequent histopathological changes detected in the skin including hyperplasia of epidermis, hypertrophy and hyperplasia of the mucosal cells and dermis edema. Some more severe alternations found in the skin of fish exposed to higher level of *A. hydrophila* (10^6 CFU/ml) included telangiectasia of dermis layer. In addition, according to the results of histometrical studies in treated fish compared to control group showed that thickness of epidermis and dermis layers were increased significantly (P<0.05).

Conclusion: *A. hydrophila* can cause major histophatological changes in the skin of *A. latus*. In addition, histopathological changes of the skin provide helpful information about the environmental conditions and as particular biomarkers may provide imminent into evaluating the general health and stress status of fish.

Keywords: *Acanthopagrus latus, Aeromonas hydrophila,* Hispathology, Skin.

IJT 2018 (1): 1-5

INTRODUCTION

Bacterial diseases in cultured fish are considered the main problem to aquaculture system in Iran. Fish farms have been facing great problems due to bacterial fish diseases that cause severe damage and mortality in Iran. The bacterium is widely distributed to aquaculture and diseases in warm water fish in Iran [1]. *Aeromonas hydrophila* is an opportunist pathogen in fish. Motile *Aeromonas* septicemia (MAS) is a more dramatic bacterial disease affecting various species of fish in both fresh and seawater and cause a serious problem for the fish farming industry in Iran as well as in other countries [2].

One of the most important benefits of using histopathological biomarkers in the environmental screening is possibility of examining specific target organs, including skin, liver and gills. They are responsible for vital functions, such as respiration, excretion, accumulation and biotransformation in the fish [3].

Fish skin is the tissue that covers the body and supports it not only from the import of pathogens and allergens, but also from the exudation of water and nutrients [4- 5]. Exposure to bacteria such as *A. hydrophila* compounds can cause number of damages in different fish organs. Skin represents important target organ suitable for histopathological assessment in searching for cells and tissue damages [6- 7].

In the present study, histopathological features were examined in the skin of *Acanthopagrus latus* after exposure to different concentrations of *A. hydrophila* over a period of 3 weeks. The aim of this investigation was to report lesions and damages in skin after an experimental exposure to *A. hydrophila* in one of the most ecologically and commercially important species of Persian Gulf, yellowfin seabream (*A. latus*).

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MATERIALS AND METHODS

Fish Maintenance and Experimental Design

Ninety immature male *A. latus* were obtained randomly from Naseri Pond (Khorramshahr, Iran), and then were acclimated for 2 week in wet lab of Khoramshahr University of Marine Science and Technology. In nine 300 L indoor tanks, containing filtered aerated pond water treated with UV. Fish were fed daily with shrimp but were starved for 48 h prior to the experiment and throughout it. Following acclimation, fish were randomly placed in nine 300 L tanks (10 fish in each tank). Tanks were then divided into one control and 2 experimental groups (each group run in triplicate). Experimental groups exposed to four concentrations of by *A. hydrophila* (10^3 CFU/ml (group 1), 10^6 CFU/ml (group 2) for 3 weeks. The average temperature= 23 ◦C, pH=7.8 and salinity= 14ppt.

Tissue Processing

The pieces of skin (with diameter of 3–5 mm), taken from of the right side in fish, and samples were fixed in 10% neutral buffered formalin. Tissue samples was then dehydrated in ascending concentrations of ethanol series, and embedded in paraffin and tick sectioned at 5µm–6µm were prepared using a RMZZ45 rotary microtome (Leica, Wetzlar, Germany). The tissue sections were stained with hematoxylin and eosin (H&E), and then microscopic evaluation was performed for histological study by light microscope using Dino lit lens (with Dino capture software, FDP2, Taiwan). [8].

Statistical Analysis

For quantitative measurements, five individuals per each tank and 5 slides from the skin of each were randomly selected for histometrical analysis. Five fields per slide were examined, each consisting of a portion of a skin epidermis and dermis layer. Epidermis thickness and dermis length were measured. All measurements were performed with light microscope using Dino lit lens (with Dino capture software, FDP2, Taiwan). All results were reported as mean ± SE. The treated groups were compared with the controls and data were analyzed using One-way ANOVA followed by Tukey's post hoc test by SPSS 16.0 software (Chicago, IL, USA).

Ethical Considerations

This research was performed according to convention of animal rights (approved by the Ethics Committee of Khoramshahr University of Marine Science and Technology). We tried to use fish without causing them unnecessary suffering if it could be avoided.

RESULTS

Histological and histometrical analysis of the skin in the control and experimental groups was conducted to assess the skin histopathological alterations resulted from exposure to different concentrations of *A. hydrophila*.

Histopathological Study

Skin did not represent any abnormality in the cell and tissue structure. The most frequent histopathological changes detected in the skin included, hyperplasia and hypertrophy of epidermis, hypertrophy and hyperplasia of the mucosal cells and dermis edema. Some more severe alternations found in the skin of fish exposed to higher level of *A. hydrophila* (10^6 CFU/ml) included telangiectasia of dermis layer (Figure. 1A-D).

Histometrical Study

The histometrical changes in epidermis and dermis layers in control and treated fish are shown in Figure 1. There were significant difference (P<0.05), in epidermis layer between concentration of (*A. hydrophila* 10^3 CFU/ml) in days 7, 14 and 21. However, no significant difference in day 3 compared to the control group (P > 0.05) (Figure 2). There were significant difference (P<0.05), in dermis layer between concentration of (*A. hydrophila* 10^3 CFU/ml) in 3, 7, 14 and 21. However, no significant difference in day 3 and 7 compared to the control group (Figure. 2).

Alteration of dermis layer affected by *A. hydrophila* concentrations of (10^6 CFU/ml) is presented in Figure 2. There were no significant difference in day 3 compared to the control group. There was significant difference between treatments fish in days 3, 7 and 14, and days 3, 7 and 21 (P<0.05). However, there was no significant difference between treatments fish in days in 14 and 21 (Figure 3).

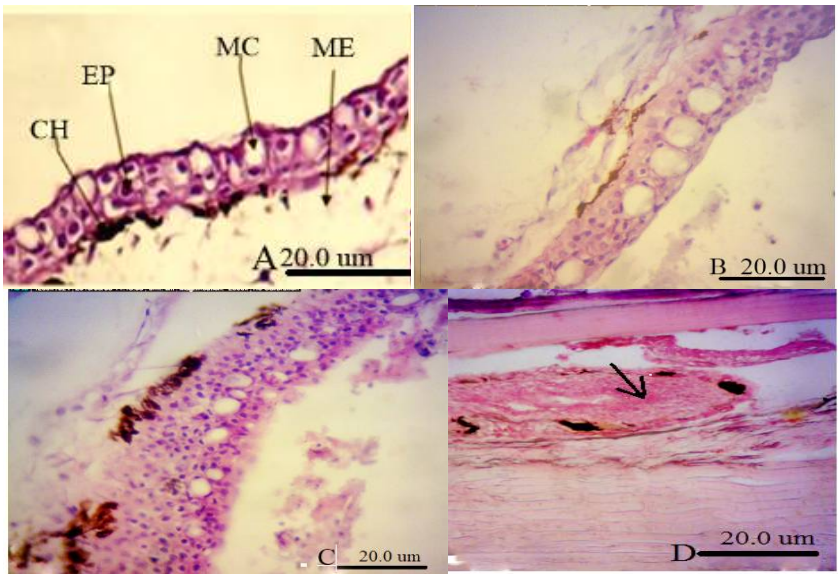

Figure 1. Photomicrographs of skin histological structure in *A. latus* . A: Normal histological structure of skin. CH: Chromatophore, EP: Epitelium cell, MC: Mucosal cell, ME: Mesanshim. (H&E); ×2900. Photomicrographs of histopathological alternations of skin within the *A. latus* groups exposed to *A. hydrophila.* (B. C, D). B: Hypertrophy of Mucosal cell. C: Hyperplasia of epitelium cells and hyperplasia of mucosal cells. D: telangiectasia of dermis layer (black arrow). (H&E); ×2900.

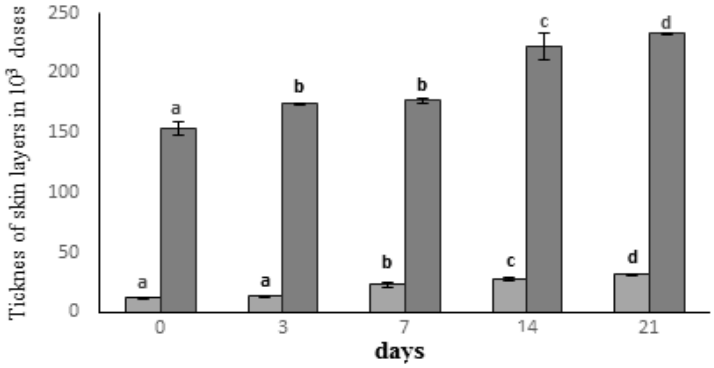

Figure 2. The effect of sub lethal concentrations of *Aeromonas hydrophila* on epidermis and dermis layer in *Acanthopagrus latus.* Data are represented as mean ± SE. The letters show the significant difference between control (day 0) and *A. hydrophila* treated groups (days 3, 7, 14 and 21).

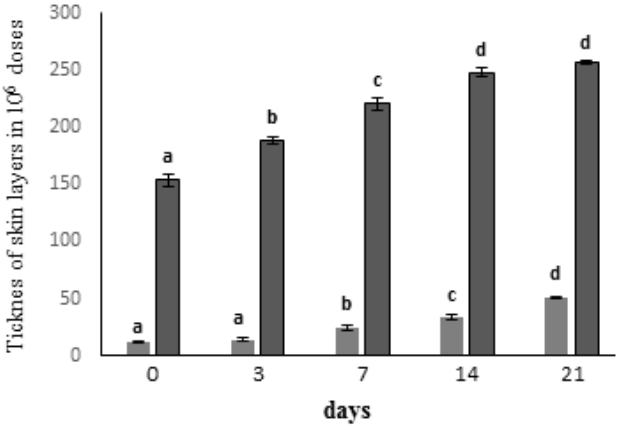

Figure 3. The effect of sub lethal concentrations of *Aeromonas hydrophila* on epidermis and dermis layer in *Acanthopagrus latus.* Data are represented as mean ± SE. The letters show the significant difference between control (day 0) and *A. hydrophila* treated groups (days 3, 7, 14 and 21).

DISCUSSION

Histopathological biomarkers have been used in fish to identify and evaluate toxic effects of exposure to pollutant [9]. The fish skin is a multifunctional organ, participates in many important functions such as protection, respiration, osmoregulation, and acid–base. Skin structure provides a large surface area for direct and constant contact with water pollutant. Thus, this organ is sensitive to chemicals and microorganisms in water, and is considered as the primary target organ to the contaminants [10- 11].

Several types of skin impairment have been documented in fish experimentally exposed to contaminants [12, 13]. It seems that, the biological characteristics of fish (such as age and sex) or seasonal factors do not affect the response of fish skin to bacterial diseases exposure. Generally, skin histopathology appears to be a promising biomarker for general environment contamination, although tissue preparation for skin histopathological study is time consuming [14].

According to our results, *A. hydrophila* could cause major histophatological changes in the skin, of *A. latus*. These changes ranged from mild to severe in this fish depending on the concentration of *A. hydrophila*. As the results showed, although *A. latus* is one of the most resistant fish species, even the lower concentrations of *A. hydrophila* influenced the normal structure of skin in this fish. The most of the histopathological alterations of skin described in the present study were in agreement with those reported in other fish species under a broad range of exposure situations, then it seems that these effects reveal physiological modification to stress rather than as special and restricted toxic responses to the concentrations of, *A. hydrophila* considered here. Changes such as hyperplasia and hypertrophy of the epithelial cells and mucosal cells, edema and telangiectasia in the dermis layer and increase of thickness in the epidermis and dermis layers, recognized in the present investigation, are usual skin lacerations in response to many other bacterial [15- 16- 17]. The major alternations in skin of *A. latus* exposed to concentrations of *A. hydrophila* in the present experiment, were a hyperplasia and hypertrophy of the epithelial cells, hyperplasia and hypertrophy of mucosal cells, edema and telangiectasia in the dermis and increase of thickness in the epidermis and dermis layers as have been reported upon exposure of mosquito fish (*Gambusia holbrooki*) to *A. hydrophila* [18- 19].

Histopathological and histometrical changes observed in the present study are similar to the responses produced by other bacteria [20- 21- 22]. Hyperplasia of epithelial cells of epidermis layer, hyperplasia and hypertrophy of mucosal cells in fish treated with *A. hydrophila* at sublethal doses 21 days are reported [23]. The same results were reported following 72 h of exposure of common carp to sublethal doses of *A. hydrophila*. Similar histopathological changes in skin are also reported [24 -25].

CONCLUSION

Histopathological changes of the skin provide helpful information about the environmental conditions and as particular biomarkers may provide imminent on evaluating the general health and stress status of fish.

ACKNOWLEDGMENTS

This manuscript results from MSc thesis (By Fatemeh Azadbakht). The authors would like to thank from Khorramshahr University of Marine Sciences and Technology for financial support. The authors declare that there is no conflict of interest.

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