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Article:

Oreochromis niloticus (nile tilapia) skin tissues response to 5hrs transportation in fresh and brackish water

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Abstract

The present investigation was carried out to study the skin histopathological changes of *Oreochromis niloticus* after 5 hours transportation stress in water with and without sodium chloride (NaCl). Three groups were used in an extreme five-hour transport mode: the control group (not transported - CG), post-transport group without salt (PT-S), and post-transport group with 5gm/L salt (PT+S). The skin tissue from the Nile tilapia PT-S group showed a flattened and thin epidermal layer with a loss of goblet cells. There was marked mononuclear inflammatory cellular infiltration around blood vessels (vasculitis). The dermal loose connective tissue lost its tight appearance and had gaps. The lower compact dermal fibrous connective tissue layer showed irregular less compact collagenous fibers. The muscular layer beneath the dermis exhibited significant interstitial myositis with inflammatory cellular infiltration. While skin tissue from the Nile tilapia PT+S group showed marked improvement in the histologic skin tissue structure including the epidermis, dermis, hypodermis and muscular layers. The improvement in epidermal layer showed normal epithelial cells and regular collagenous bundles of dermal layer and normal hypodermis. Conclusion: Our findings have significant importance in the field of fish aquaculture and address the importance of skin and its mucous cover health during transportation, we recommend the use of salt during transport of O. niloticus as the benefits of it using during transport appear to reduce the effects of transport stress.

Keywords: *Skin, NaCl, Transportation, Stress, Nile tilapia O. niloticus, Histopathology, Sodium chloride.*

Introduction

Aquaculture and fisheries industries have several unavoidable stressors, such as handling, transportation, temperature, crowding, salinity that result in the stress responses of fish (Eissa and Wang, 2016). The transport of live fish is a common practice in aquaculture (Harmon, 2009 and Vanderzwalmen et al., 2019). Transport includes several steps whether pre-transport such as collection, grading, netting, air exposure, and packing and during transport process such as water movement, vibrations, and water condition change, which are stressful to fish (Pakhira et al., 2015). The skin, with its scales and surface mucus provides protective physical barrier that is important in terms of both osmoregulation and pathogen defense. But fish skin is susceptible to damage from handling, fighting, physical trauma, predation, environmental irritants and pathogens. At that stage, the stress response may further compromise the host's defenses, via corticosteroid mediated immune-suppression or other stress-related immunosuppressive factors (Choi et al. 2007).

Harmon (2009) and Vanderzwalmen et al. (2019) reviewed common salt in the transport water of fish had an immediate reduce stress action. The use of 5 g of salt L−1 transport water of rainbow trout (Onchorynchus mykiss) was efficient in maintaining the integrity of the skin's mucosa and microbiota, as well as due to its ability to inhibit the growth of Vibrio anguillarum (Tacchi et al.,

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2015). The aim of the present study was to examine the *Oreochromis niloticus* skin histopathological responses to 5hrs transportation stress in water with and without sodium chloride (NaCl – salt).

Materials and Methods

Ethical Considerations: Animal handling and rights have been approved by the Veterinary Medical Research Ethics Committee, Faculty of Veterinary Medicine, Sohag University, Sohag, Egypt (Approval number: Soh.un.vet /00038).

Experiment design and sample collection: Sixty Nile tilapia (*Oreochromis niloticus*) with an average weight of 100±10g were obtained from the tilapia breeding farm at Assiut province. The tested fish were subjected to 5hrs transportation; three transport groups. The 1st fish group was control group (CG) that sampled at the farm site without transportation, the 2nd group was PT-S group and sampled post 5 hours of transport stress and the 3rd group was PT+S group that transported for 5hrs in water contains 5g/L NaCl. Transport water was taken directly from the farm's pond and the fish were transported by truck for five hours without sedative to the Wet Laboratory of Fish Diseases and Management Department - Faculty of Veterinary Medicine, Sohag University. The fish skin tissues were sampled before (control group) and after 5 hours of transport stress in the PT-S and PT+S groups. Twenty fish were sampled from each group. Fish were anesthetized with MS-222 prior to skin sampling then the fish are euthanized by transection of the spinal cord, the skin samples have dissected and preserved in 10% neutral buffered formalin for histopathological examination.

Tissue preparation for histopathological examination: For staining with haematoxylin-eosin(HE), skin samples from three fish groups fixed in 10% neutral buffered formalin were used. After fixation the specimens were dehydrated in ascending grade (70, 80, 90, and 100%) of ethyl alcohol for one hour in each of the first three concentrations, while two changes of 100% ethyl alcohol were used for half an hour each. The specimens were then cleared in xylene for 20 minutes. The specimens were then embedded in paraffin wax for one hour and sectioned at 5-7 µm thickness. For staining; sections were deparafinized by impeding in xylene for 1-24 hr. with shaking; and then hydrated in descending grades of ethyl alcohol till 50% and then in distilled water for 5 minutes for each change. Sections were stained in haematoxylin stain for one minute then washed in running tap water. The excess of the stain was removed by washing in 0.5%-1% hydrochloric acid in 70% ethyl alcohol for a few minutes, and then by distilled water. Subsequently, the sections were stained in eosin for 5 minute, then passed in 95 -100% of ethyl alcohol, clarified in xylene, mounted by Canada balsam and then examined microscopically using OLYMPUS CX43 with adapted camera OLYMPUS P72(department of pathology and clinical pathology -Faculty of Veterinary Medicine, Sohag University) as previously described by Drury and Wallington (1980).

Results

The Skin sections from the control fish group (CG group) showed normal structured outer epidermis composed from multilayered flattened epithelial cells with goblet cells and melanocytes, the basement membrane is found between the epidermis and dermis melanocytes filled with melanin pigment. Some blood vessels are recognized in the dermal layer. The dermis is composed mainly of tight fibrous connective tissue, comprises a thin upper layer (loose connective tissue) and a thick dense layer (stratum compactum) Figure (1). While the skin tissue from Nile tilapia PT-S group showed flatten and thin epidermal layer with lost goblet cells, marked mononuclear inflammatory cellular infiltration around blood vessels (vasculitis); dermal loose connective tissue loss its tight appearance with the presence of gabs, lower compact dermal fibrous connective tissue layer showed irregular less compact collagenous fibers. Muscular layer under dermis showed marked interstitial myositis with inflammatory cellular infiltration Figure (2). Skin tissue from Nile tilapia PT+S group showed marked improvement in histologic skin tissue structure including Epidermis, Dermis, Hypodermis, Muscular layers (M), maintaining the integrity of the skin's mucosa and improvement in epithelial cells of epidermal layer and regular collagenous bundles of dermal layer, normal hypodermis Figure (3)

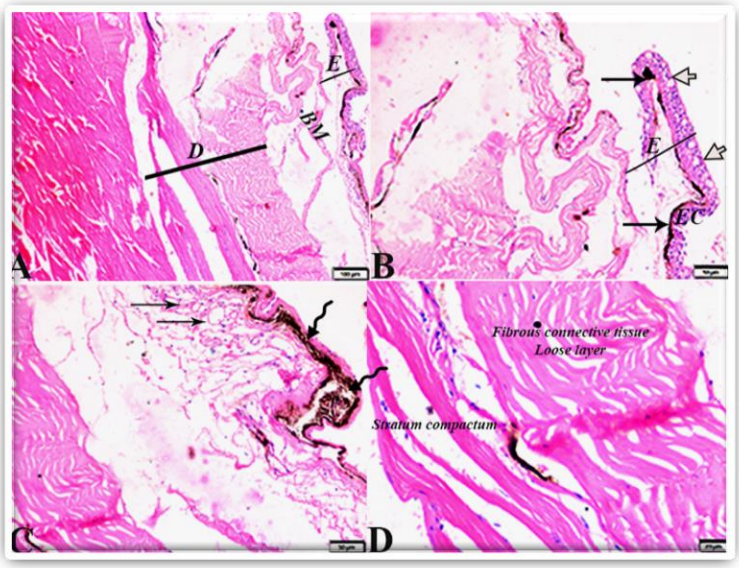

Figure 1 Photomicrograph of skin tissue from Nile tilapia control group (CG) showing (A magnified in B): normal structured outer epidermis (E) composed from multilayered flattened epithelial cells (EC) with goblet cells (white arrowheads), Melanocytes (arrows). A basement membrane (BM) is found between the epidermis and dermis. (C): melanocytes filled with melanin pigment (zigzag arrows). Some blood vessels are recognized in the dermal layer (arrows). (D): The dermis is composed mainly of tight fibrous connective tissue, comprises a thin upper layer (loose connective tissue) and a thick dense layer (stratum compactum). (HE stains, the bar size was 50µm).

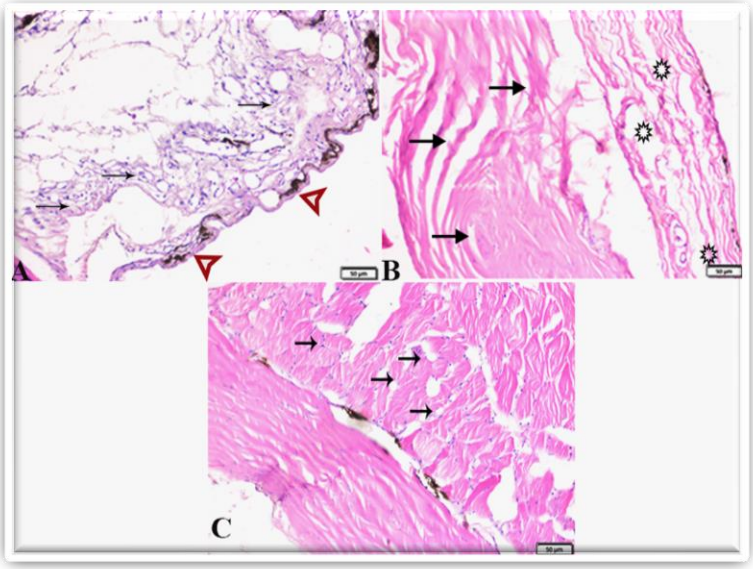

Figure 2 Photomicrograph of skin tissue from Nile tilapia PT-S group showing (A): flattened and thin epidermal layer with lost goblet cells (arrowhead), marked mononuclear inflammatory cellular infiltration around blood vessels (vasculitis) (arrows). (B): dermal loose connective tissue loss its tight appearance with the presence of gabs (stars), lower compact dermal fibrous connective tissue layer showed irregular less compact collagenous fibers (arrows). (C): muscular layer under dermis showed marked interstitial myositis with inflammatory cellular infiltration (arrows). (HE stains. The bar size was (A=100µm, B&C=20 µm).

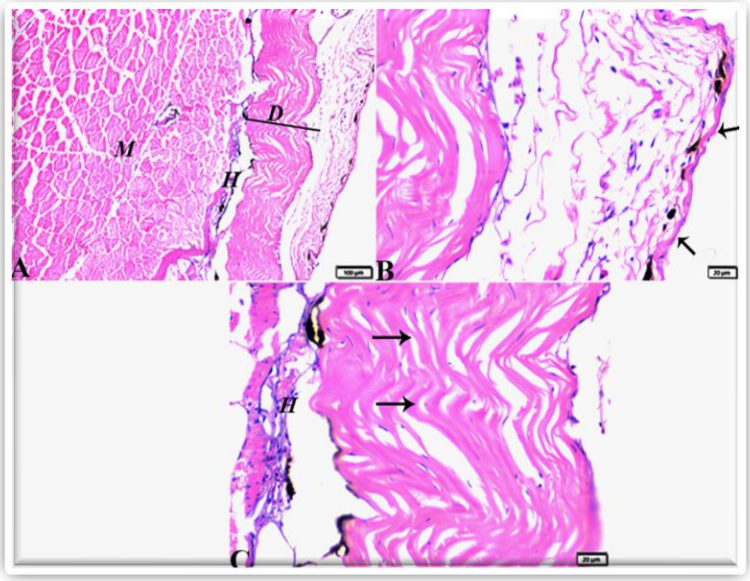

Figure 3 Photomicrograph of skin tissue from Nile tilapia PT+S group showing (A): marked improvement in histologic skin tissue structure; Epidermis (E), Dermis (D), hypodermis (H), muscular layer (M). (B): improvement in epidermal layer epithelial cells (arrows). (C): regular collagenous bundles of dermal layer (arrows), normal hypodermis (H). (HE stains. The bar size was (A =100µm, B&C=50 µm, D=20 µm).

Discussion

Transportation of live fish is an essential step for fish aquaculture industries including transportation of fish from one farm to another, during restocking practices, from hatcheries to farms, rivers, lakes. It involved both food and companion fishes and associated with mechanical and water quality deterioration stress on transported fishes (Sampaio and Freire, 2016). Therefore, objective criteria to assess the health and welfare of the fish are increasingly required (Varsamos et al. 2006).

Skin histopathology of O. niloticus in PT-S group showed flatten and thin epidermal layer that may be due to injuries arising during preparatory operations for transport and to aggressive actions between fish that induced by stress, resulting in the loss of protective mucus coat that increased risk of injury. This explanation was supported by the finding of Harnish et al., (2011) and Tacchi et al., (2015). In addition, the skin histopathology of O. niloticus in PT-S group showed the absence of goblet cells. Similar findings were consistent by Zheng et al., (2021) who reported that the number of mucus cells decreased after 8 hours of transport stress. This result may be attributed to the alterations changes in water conditions, which may also affect the number of epidermal goblet cells, also the skin mucus cell counts could be used as an indicator of exposure to a stressor known to involve continuous secretion and shedding of mucus produced by skin mucus cells (Iger and Abraham (1997). Oppositely, Van Der Mare et al. (2010) and Vatsos et al. (2010) found an increased number of mucus cells in the skin in response to external stressors. This difference may be attributed to various stress conditions and the unclear physiological mechanism that leading to a change in the number of skin mucosal cells.

Concerning the histopathological study of PT+S group skin sections recorded marked improvement in histologic skin tissue structure including the epidermis, dermis, hypodermis, muscular layers and the 5mgL-1 was efficient in maintaining the integrity of the skin's mucosa. Slight anti-inflammatory effect was noted in the skin tissues of the PT+S group. These results may be attributed to the fact that the salt has a stress-reducing effect by acting as a regulator of mucus release from goblet cells and reducing the energy requirements for osmoregulation and homeostasis through reducing the difference between the internal and environment osmolality (Nikinmaa et al. (1983) and adding salt to transport water was effective in maintaining skin integrity (Tacchi et al., (2015).

These results indicated that O. niloticus in fish PT-S group was subject to a greater transport stress effect and microbial invasion than the other two groups, and the addition of salt to the transport water mitigated the transport stress and reduced the risk of bacterial invasion by increasing the production of antimicrobial peptides and anti-inflammatory effects, improved mucus

properties, particularly viscosity, capture capacity and physical barrier, restored the mineral osmotic balance between fish and water, and preserved the integrity of the skin's outer surface.

Authors' contribution

The work was equally distributed between authors, Mohamed Abd El Aziz Ahmed Abd El-Galil, Mousa A. Mohamed and Hana N. Heba contributed to the sample collection, designed the research study, arranged the images and wrote the paper, Abd El-Lateif S. Rasha, Seddek A M contributed to the final editing and revision of paper. All authors have read and approved the final version of the manuscript.

Conflict of interest

The authors declare that there was no conflict of interest for the publication of this article.

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