Received: 11 March 2023, Accepted: 17 April 2023

Original Research

Journal of Advanced Veterinary Research
(2023) Volume 13, Issue 3, 360-367

Effect of Water Quality on Tilapia Microbiota and its Reflection on Health Status

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Abstract

Fisheries and aquaculture are the main players in world food security and nutrition as they constitute major sources of animal protein. We aimed to evaluate the effect of water quality on microbiota and its reflection on the health status of Nile tilapia (*Oreochromis niloticus*). One hundred Nile tilapia and 60 water samples were collected from the Ismailia canal, Bahr Mowais, river-irrigated aquacultures, and Agriculture drainage irrigated aquacultures. The real-time PCR results indicated that the water as well as the gills and intestine of fish from the Ismailia canal had the lowest load of opportunistic bacteria while Agriculture drainage irrigated aquaculture had the highest load. The prevalence of *Aspergillus niger* and *Aspergillus flavus* was higher in tilapia from aquacultures. The gills of fish from aquaculture groups showed hyperplasia and telangiectasia while their spleen showed activated melanomacrophage centers with diffuse leucocytic infiltration and their intestines showed increased villus width and length. The blood of the aquaculture groups showed a decline in hematological parameters, phagocytic activity, IgM values, and leucocytic counts while their neutrophil content was elevated. We concluded that the bad water quality affected the health and immune status of tilapia directly and indirectly by rising the pathogenic load.

KEYWORDS
Water quality; Nile tilapia; microbial load; Ammonia toxicity; Activated melanomacrophage center; Immunity impairment.

INTRODUCTION

Fisheries and aquaculture are the main players in world food security and nutrition as they constitute major sources of animal protein. Furthermore, fish provides Egyptian with almost 20 percent of their average per capita intake of animal protein. Moreover, Nile tilapia (*Oreochromis niloticus* (Linnaeus, 1758)) represents the main species produced in Egypt (FAO, 2020).

The aquatic ecosystem is dynamic and several ecological factors and biological agents surround the aquatic organisms. In fish, the variations in water parameters directly affect the pathogenic loads and primary barriers including skin, gills, and gut as well as the internal environment (Sundh and Sundell, 2015). Moreover, skin, gills, and gut are the major pathways for pathogen entry in fish (Ringø *et al*., 2007; Bøgwald and Dalmo, 2014)

Approximately 75% of the feed nitrogen and phosphorus are not utilized and remain as wastes in the water, thus affecting water quality (Li *et al*., 2019) which directly and indirectly affects fish health and immune status. Directly, via the production of toxic products in particular, ammonia which can irritate or damage the gill epithelial cells and compromise the immune systems of fish (Roberts, 2012), or biodegraded to highly toxic nitrite which increases the level of methemoglobin in the blood, resulting in tissue hypoxia, nerve palsy, or even suffocation and death (Tilak *et al*., 2007). While indirectly, the increased nitrogen and phosphorus increase the algae and aquatic plants, which consume oxygen during the night causing environmental hypoxia in the early morning (Noga, 2010), which could alter the microbiota composition (Ni *et al*., 2018).

The beneficial microbiota present on the mucosal surfaces is a key component of the host mucosal immunity by antagonizing the pathogenic one through competition for nutrients and colonization sites as well as, the production of inhibitory compounds (Merrifield and Rodiles, 2015). Noteworthy, disrupting the integrity of this critical defense mechanism can increase the risk of infection (Khosravi and Mazmanian, 2013). Therefore, the increased load of the genus including important fish pathogens enhances the risk of opportunistic infection. The major causal agents of bacterial diseases in tilapia in Egypt are *Pseudomonas* spp., *Aeromonas* spp., and *Streptococcus* spp. (Zahran *et al*., 2016). *Aspergillus* spp. is a normal microflora of Nile tilapia and possesses virulence factors, so under unfavorable conditions, it may cause diseases (Refai *et al*., 2010).

Real-time PCR (RT-PCR) increased the ability to quantify the

ISSN: 2090-6277/2090-6269/

organisms in samples accurately due to high analytical sensitivity and specificity with confidence in the microbe identity (Merrifield and Rodiles, 2015; Borchardt *et al*., 2021). Moreover, cycle threshold (CT) values inversely relate to the number of copies of the target gene in a sample, meaning that lower CT values correlate with higher pathogen loads (Bonacorsi *et al*., 2021). In addition, Jeong *et al*. (2022) found that the relative proportion in 100 people calculated from the NGS frequency was almost similar to that based on RT-PCR CT value. This suggests that the CT value of RT-PCR, without calibration, can indicate relative bacterial load.

This study aimed to evaluate, under actual field conditions, the effect of water quality on pathogenic microbial load and its reflection on the health status of tilapia. In this study, we tried to dissect factors that shaped the microbiota as an initial step toward predicting fish diseases.

MATERIALS AND METHODS

Ethical Approval

The Institutional Animal Care and Use Committee (ARC-IACUC) at the agriculture research center approved this study under number (ARC/AH/22/12).

Sampling sites

This study included two types of water systems the river and aquaculture. Sampling from the river included Bahr Mowais (BM), and Ismailia canal (IC). Sampling from aquaculture consisted of 10 ponds from Abbassa including five ponds irrigated with river water (RI) and another five irrigated with agriculture drainage water (ADI) (Fig. 1).

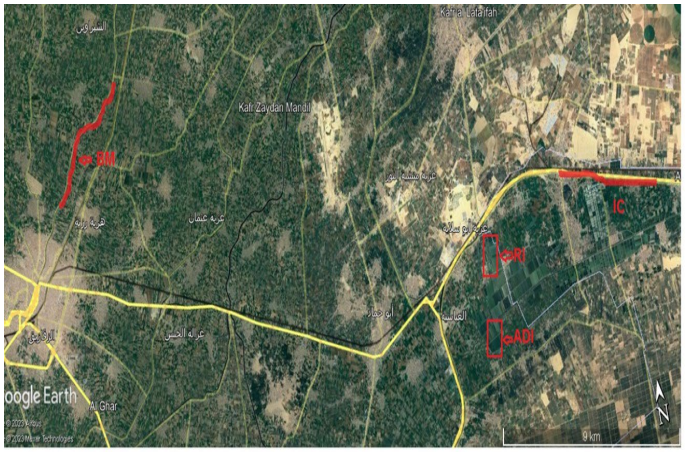

Fig. 1. Sampling sites of Nile tilapia.

Water sampling and measurement

Water and fish samples were obtained in the morning (8 – 9 am) during May and June. Three water samples were collected from each pond and three samples from each location from each river branch. The locations within the same branch are one km away from each other. Water samples were collected at 20 cm below the surface. Firstly, a 500 mL sample was collected in polyethylene bottles for physical and chemical analyses. Total dissolved solids (TDS), pH, temperature, and Dissolved oxygen (DO) were measured in situ using a hand-held YSI meter (YSI, Yellow Springs, OH, USA). Nitrite, ammonia, and phosphate were determined according to APHA (1995).

Secondly, a 500 mL sample was collected in sterile glass bottles for microbiological analysis, where, 100 ml was directed for fungal isolation and identification, and another 100 ml was stored at -20°c for the following examination. Samples were properly labeled and placed in an icebox filled with ice until reaching the lab. of the Animal Health Research Institute, Zagazig branch.

Fish sampling

From each sampling site in the river and each pond, we collected and transported five healthy Nile tilapia alive in a tank filled with water from its sampling site to the wet lab. The fish were euthanized with an overdose of tricaine methanesulfonate (MS222) (Sigma-Aldrich, St Louis, MO, USA). Then 1 cm of gills and 1cm of foregut from each fish were dissected and homogenized in 10 ml buffer saline and stored at -20°c for future use.

Fungal Isolation and Identification

Homogenized 1 cm of gills and intestine in 10 ml sterile water and 100 ml from each water sample were diluted to 1/1000. Then 1 ml from each dilution was aseptically inoculated into Sabouraud's dextrose agar supplemented with Rose Bengal (1/15,000) and 50 ppm chloramphenicol (Garrett, 1981; Smith and Dawson, 1944) and incubated at 27˚C for 5 to 7 days (three plates for each sample). Developing colonies were identified using the identification key of Raper and Fennell (1965) for *Aspergillus*.

Bacterial DNA preparation

DNA extraction of the stored samples (pooled) was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH, Catalogue no.51304) with primer pairs presented in Table (1). Briefly, 2 µl of the extracted DNA (Josefsen *et al*., 2010) was added to 18 µl of the master mix (iQ™ SYBR Green Supermix; Bio-Rad, USA). The cycling parameters were initial denaturation at 95°C for 10 minutes followed by 35 cycles of denaturation at 95°C for 45 seconds, annealing at 52°C for 45 seconds, and extension at 72°C for 10 minutes with the final holding temperature at 4°C. The fluorescence intensity of SYBR Green and the melting curve analysis were evaluated and CT under 35 was considered a positive result.

Blood samples

Each group had three different types of pooled blood samples taken from the caudal vein under strict aseptic conditions: A) 1 ml on EDTA for hematological analysis. B) 2 ml of heparin in

Table 1. Primers used for SYBR Green RT-PCR assays.

Target gene	Sequence (5'-3')		Reference
	F	R	
Aeromonas Aerolysin (M16495)	CCAAGGGGTCTGTGGCGACA	TTTCACCGGTAACAGGATTG	Pollard *et al*. (1990)
Streptococcus (*tuf*)	GTACAGTTGCTTCAGGACGTATC	ACGTTCGATTTCATCACGTTG	Picard *et al*. (2004)
Pseudomonas (*opr*I)	AGCCTTCCTGGTCCCCTTAC	CCTAATGAACCCCAGTGTATAAGTTTG	De Vos *et al*. (1997)

a sterile plastic tube for analyzing phagocytic activity. C) 3 ml in a centrifuge tube that has been thoroughly cleaned and dried, allowed to clot at room temperature, and then centrifuged at 3000 rpm for five minutes. For biochemical analysis, serum was drawn out, labeled, put in dry, clean tubes with caps, and frozen at -20°C.

Hematological and biochemical study

RBCs and differential leucocytic counts were determined according to Feldman *et al.* (2000) and (Cole, 1986) respectively, while hemoglobin concentration (Hb), packed cell volume (PCV), and total leucocytic count manually (Blaxhall and Daisley, 1973). The estimation of Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, the serum urea, creatinine, and Immunoglobulin M (IgM) were according to Murray (1984); Fawcet and Scott (1960); Henry (1974), and Naito (1986) respectively.

Phagocytic activity and index

The phagocytic activity of Peripheral Blood Monocytes was measured using Candida albicans (Anthony *et al.*, 1985; Chu and Dietert, 1989). Peripheral Blood Mononuclear Cells were separated using ficoll-plaque density gradient (Boyum, 1986; Goddeeris *et al.*, 1986). and then the Phagocytic activity and index were assayed (Wilkinson, 1976).

Histopathological examination

Tissue specimens collected from the gills, spleen, and intestine were fixed in 10% buffered neutral formalin solution, dehydrated in gradual ethanol (70-100%), cleared in xylene, and embedded in paraffin. 5µm thick paraffin sections were prepared and then routinely stained with hematoxylin and eosin (H&E) stains for the microscopical examination.

Intestinal measurement

Each section of the intestine was examined to count the goblet cells (GCC), villi length (µm), and villi width (µm) using a micrometer slide according to Kuitunen *et al.* (1982). Results were demonstrated photomicrography in obtained figures and statistically analyzed according to Weibel (1963).

Statistical analysis

The obtained data were analyzed by one-way ANOVA and then Duncan's Multiple Range (Duncan, 1955) at a significance level of 0.05 using the SPSS program SPSS (2004).

RESULTS

Table 2 shows the measured water parameters from different sources. The DO was highest in IC and lowest in ADI while it contained the highest values of pH, TDS, ammonia, nitrite, and phosphate.

The prevalence of *Aspergillus niger* was higher in ADI; while *Aspergillus flavus* was higher in RI, BM, and IC as shown in Table 3.

Figures 2, and 3 explore the CT values of *Streptococcus* spp. and *Aeromonas* spp. in water and gills and intestine of Nile tilapia from different sources where the ADI had the lowest values while IC showed the highest values. The CT values of *Pseudomonas* spp. in tilapia intestines from different sources were nearly the same, while in gills and water, the CT values were higher in the wild than in the cultivated as shown in Fig. 4.

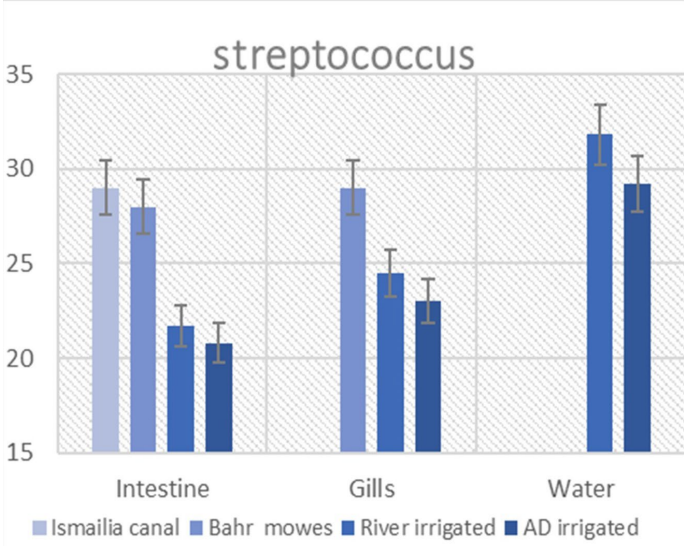

Fig. 2. CT values of *Streptococcus* spp. in water and intestine and gills of Nile tilapia from different sources.

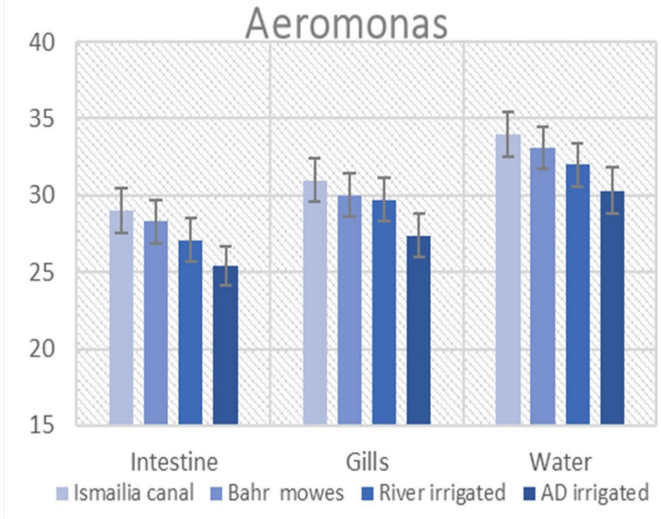

Fig. 3. CT values of *Aeromonas* spp. in water and intestine and gills of Nile tilapia from different sources.

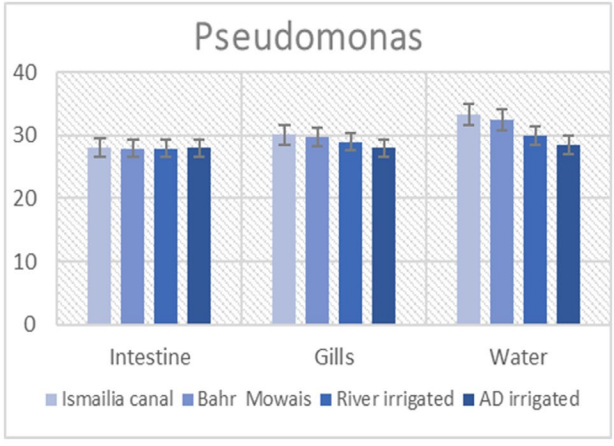

Fig. 4. CT values of *Pseudomonas* spp. in water and intestine and gills of Nile tilapia from different sources.

Results of hematological parameters represented in Table 4 showed a significant decrease (P<0.05) in the RBCs count, Hb concentration, and PCV in RI and ADI with leukopenia, lymphopenia, neutrophilia, and monocytopenia compared to IC and BM fish.

Results of the biochemical parameters revealed a significant increase (P<0.05) in AST, ALT, creatinine, and urea in RI and ADI compared to IC and BM (Table 5).

Results of immunological parameters represented in Table 6 showed significant decreases (P<0.05) in phagocytic ratio, phagocytic index, and IgM in RI and ADI compared to IC and BM.

Concerning the histopathological results, Fig. 5 showed the gills of Nile tilapia from BM showed hyperplasia of lamellar epithelium and mild telangiectasia (Fig. 5a), while those from RI revealed curving of secondary lamellae, severe hyperplasia of lamellar epithelium, destructed gill filaments, and fusion of the

Table 2. Water quality parameters of different water sources.

	Ismailia canal	Bahr Mowais	River irrigated	AD irrigated
Temperature °C	27.7±0.40a	27.8±0.30a	27.9±0.38a	28.1±0.40a
DO (mg L^{-1})	7.88±0.22a	7.7±0.29a	5.4±0.510b	5.22±0.70b
pH	7.29±0.10b	7.43±0.05b	8.22±0.26a	8.42±0.23a
TDS (mg L^{-1})	244.0±14.0c	289.0±29.0c	380.0±30.0b	756.0±51.0a
Ammonia (mg L^{-1})	0.12±0.04c	0.18±0.03c	0.31±0.04b	0.52±0.06a
Nitrite (mg L^{-1})	0.01±0.004d	0.02±0.004c	0.04±0.0065b	0.066±0.007a
Phosphate (mg L^{-1})	0.067±0.014c	0.116±0.017c	0.411±0.037b	0.651±0.061a

Group with different letters within the same row are significantly different at P < 0.05.

Table 3. Prevalence of *Aspergillus* spp. in Nile tilapia and water from all sources.

	Type of mold	Ismailia canal	Bahr Mowais	River irrigated	AD irrigated
Water	*Aspergillus flavus*	0	1(20%)	4(27%)	5(35%)
	Aspergillus niger	0	0	3(21%)	6(40%)
Gills	*Aspergillus flavus*	2(8%)	2(8%)	6(24%)	8(32%)
	Aspergillus niger	1(4%)	2(8%)	5(20%)	9(36%)
Intestine	*Aspergillus flavus*	5(20%)	8(32%)	10(40%)	12(48%)
	Aspergillus niger	3(12%)	6(24%)	10(40%)	15(60%)

Table 4. Mean values of hematological parameters in Nile tilapia.

Groups	Ismailia canal	Bahr Mowais	River irrigated	AD irrigated
RBCs (×10^6 /µl)	4.22±0.10a	4.15±0.23a	2.86±0.25b	2.11±0.17c
Hb (g/dl)	11.81±0.40a	10.19±0.39b	8.76±0.21b	6.83±0.70c
PCV (%)	34.46±0.20a	32.80±0.75a	25.46±1.9b	18.92±1.70c
WBCs (×$10^3$$mm^{3)}$	8.85±0.74 ab	10.18±0.88a	7.28±0.23bc	5.68±0.17c
Lymphocytes (×$10^3$$mm^{3)}$	6.14±0.16a	6.81±1.10a	3.15±1.10b	2.64±0.65b
Neutrophils (×$10^3$$mm^{3)}$	2.15±0.57b	2.41±0.14b	3.16±0.58a	2.7±0.91a
Monocytes (×$10^3$$mm^{3)}$	0.41±0.35a	0.42±0.26a	0.32±0.15a	0.18±0.11b

RBCs: Red blood corpuscle; Hb: Hemoglobin; PCV%: Packed cell volume; WBCs: White blood cells.
Group with different letters within the same row are significantly different at P < 0.05.

Table 5. Mean values of biochemical parameters in *Oreochromis niloticus*.

Groups	Ismailia canal	Bahr Mowais	River irrigated	AD irrigated
ALT (U/L)	19.20±1.9b	17.56±1.6b	38.36±1.8a	34.7±1.2a
AST (U/L)	67.18±4.1c	72.3±5.1bc	83.8±3.0b	111.75±4.1a
Creatinine (mg/dl)	0.50±0.05b	0.45±0.03b	0.63±0.02a	0.71±0.1a
Urea (mg/dL)	18.18±1.3c	19.9±2.3c	32.85±1.3b	40±2.4a

AST: aspartate aminotransferase; ALT: alanine aminotransferase
Group with different letters within the same row are significantly different at P < 0.05

Table 6. Mean values of immunological parameters in *Oreochromis niloticus*.

Groups	Ismailia canal	Bahr Mowais	River irrigated	AD irrigated
Phagocytic ratio	57.66±1.4a	61.0±2.3a	44.66±3.1b	40.62±1.7b
Phagocytic index	2.3±0.19a	2.4±0.1a	1.6±0.2b	1.0±0.2c
IgM	432.0±17.2a	415.0±13.4a	269.0±25.4b	194.0±11.8c

Group with different letters within the same row are significantly different at P < 0.05

secondary lamellae with some round cells infiltration (Fig. 5b). Lastly, those from ADI suffered severe lamellar congestion, telangiectasia, marked necrosis of gill filaments, and exudative inflammatory exudate in the gill arch (Fig. 5c).

Fig. 6 demonstrates that the spleen of the Nile tilapia BM showed few aggregates of melanomacrophage centers (MMCs) and mild focal depletion (Fig. 6a), while fish from RI explored mild activation of MMCs and moderate depletion of the splenic pulps (Fig. 6b). Concurrently, fish from ADI showed diffuse activation of MMCs (Fig. 6c).

Fig. 7, shows the Villus length (VL) detected in those samples of RI followed by ADI were significantly higher than IC and BM. Villus width (VW) was the maximum average in RI and BM. While GCC was significantly higher in ADI followed by RI then BM and IC.

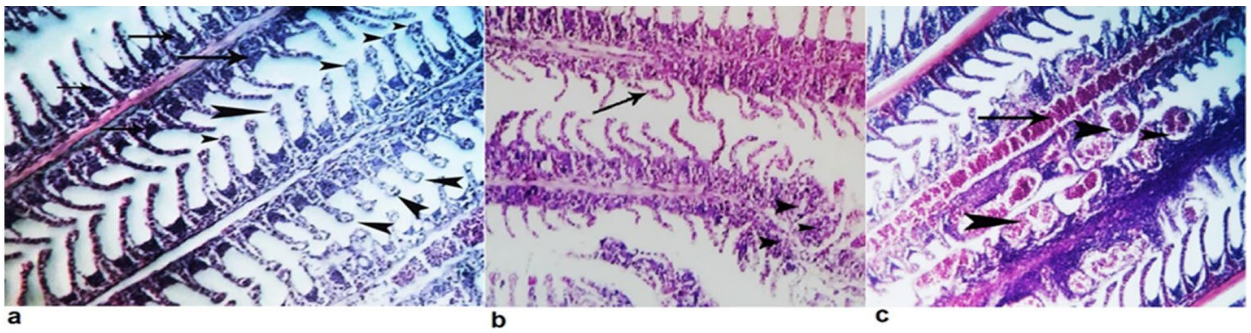

Fig. 5. Gills of Nile tilapia from a) BM showing hyperplasia of the lamellar epithelium (arrows) and mild telangiectasia (arrowheads) (H&E x400). b) RI showing curving of some secondary lamellae(arrow), severe hyperplasia of the lamellar epithelium (arrowhead), and fusion of some secondary lamellae (H&E x 300). c) ADI showing severe lamellar congestion (arrow), necrosis and fusion of the secondary lamellae, and severe telangiectasia (arrowhead) (H&E x 400).

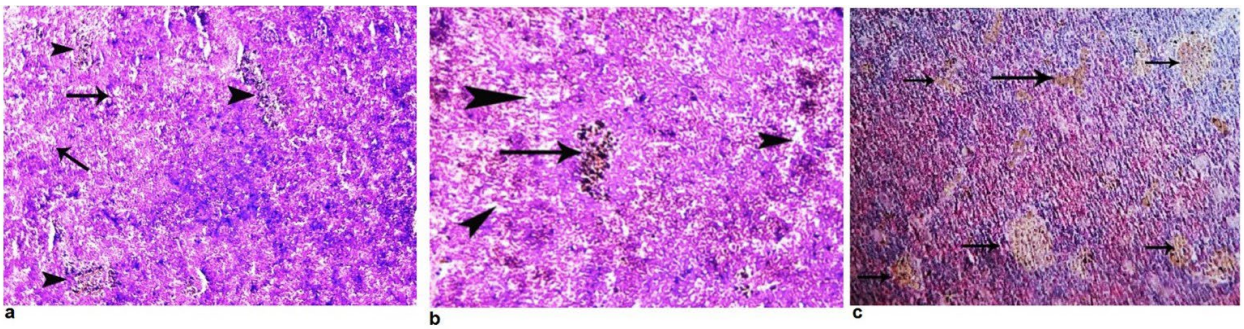

Fig. 6. Spleen of Nile tilapia from (a) BM showing mild activated MMCs (arrowheads) and focal depletion (H&E x 200). b) RI showing moderate activation of MMCs (arrow) and moderate depletion of the splenic tissues (arrowheads) (H&EX200). c) ADI showing diffuse activation of MMCs (arrows) (H&E x 200).

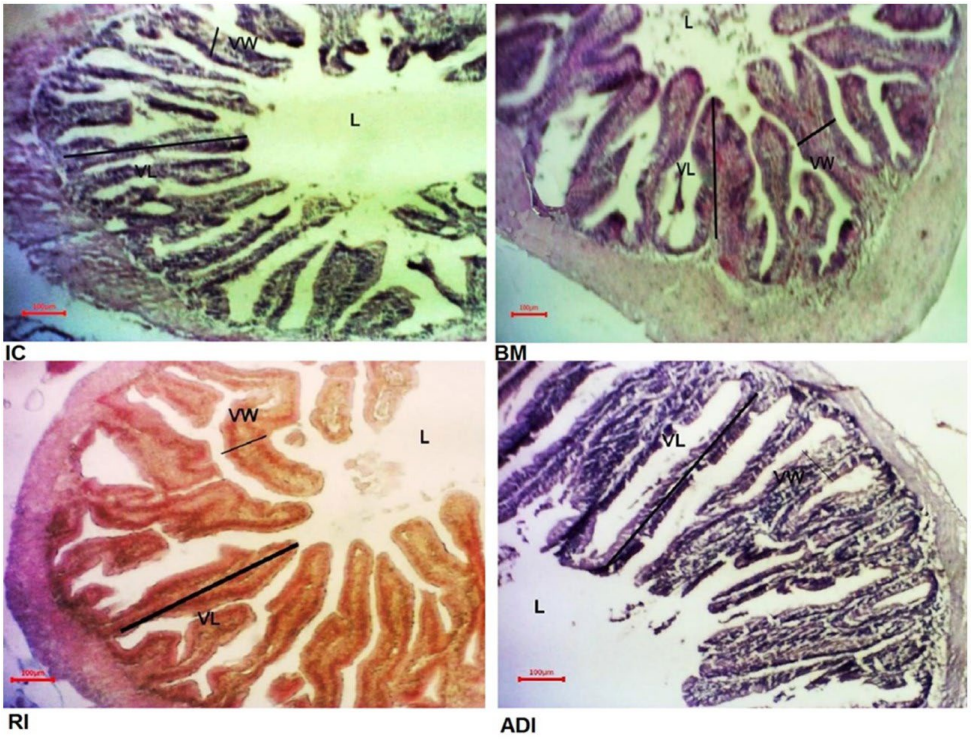

Fig. 7. Intestinal measurement of Nile tilapia from different sources.
VL= Villus length, VW=Villus width, L=Lumen

Table 7. Intestinal measurements and goblet cell count of Nile tilapia from different sources (Parameters /10 villi).

Groups	Ismailia canal	Bahr Mowais	River irrigated	AD irrigated
Villus length (VL)	414.45±24.1b	493.65± 30.9b	638.67± 52.2a	622.84± 36.7a
Villus width (VW)	69.53± 3.3c	94.27± 2.8a	91.23± 3.6ab	81.84± 3.7b
Goblet cell count (GCC)	5.2± 0.41d	9.8± 0.8bc	18.8± 4.5b	39.06± 7.5a

Means with different letters at the same row were significant P<0.05.

DISCUSSION

The microbiota in healthy individuals is in a homeostatic balance between the beneficial and the potentially pathogenic (Bien *et al*., 2013). Overgrowth of this later is an indicator of microbiota homeostasis disruption (DeGruttola *et al*., 2016). Certain water parameters when outside the preferable levels can stress the fish, impair their immunity, disrupt the microbiota composition, and increase the risk of infection.

In water, the availability of nutrients is the key to the microbial selection regime (Attramadal *et al*., 2014). Our results of the aquacultures showed higher loads (lower CT values) of the opportunistic bacteria as well as increased occurrence of *Aspergillus* spp. The accumulation of the feed waste and feces caused an increment in the organic matter, which become a bacterial and fungal substrate, thus favoring R-selection. While waters from rivers showed lower loads of the potentially pathogenic microbiota. The scarcity of the nutrient supply per bacterium and fungus in river waters pushes them toward the K-selection.

The main microbial components of the gills of Nile tilapia were similar to the surrounding water (Al-Harbi & Uddin, 2005). Our results of the microbiome on the gills of Nile tilapia from IC and the BM indicated lower loads of opportunistic pathogens, which may be attributed to the continuous water current passing over the gills (Mudarris and Austin, 1988) besides, the presence of beneficial bacteria with antibacterial properties on the gills (Ringø and Holzapfel, 2000) that resisted the colonization of pathogenic microbes. Our histopathological findings support this explanation, where the gills of fish from IC was normal while those from BM showed mild hyperplasia of secondary lamellar epithelium and mild telangiectasia.

On contrary, the gills of Nile tilapia from RI showed curving of some secondary lamellae, severe hyperplasia, destructed gill filaments, and round cells infiltration while those from ADI suffered severe lamellar congestion, telangiectasia, marked necrosis of gill filaments, exudative inflammatory cells in the gill arch. Moreover, Olaniyi *et al*. (2018), Liu *et al*. (2021), and Mangang and Pandey (2021) showed similar changes in the gills of fishes exposed to ammonia. We observed that the degree of the pathology in gills was interrelated to the levels of ammonia and pH of the surrounding water. Noteworthy, even low levels of unionized ammonia (0.02mg/l) could cause branchial hyperplasia as a defense response of the fish against waterborne irritants (Roberts, 2012). Furthermore, the variations in water parameters directly affect the microbiome of aquatic species (Sylvain *et al*., 2016) by changing the water microbiome. Concurrently, we found a higher load of opportunistic pathogens on the gills of tilapia from ADI and to a lower extent in IR in our study. We suggested that the lamellar hyperplasia provided a protective area from the water current allowing bacterial and fungal colonization, while the congestion, telangiectasia, and the marked necrosis of gill filaments performed a good substrate for their uncontrolled growth, and so, disrupted the normal microbiota on this mucosal surface.

Sylvain and Derome (2017) supposed the horizontal enrolling of fish gut microbiota symbionts from the environment (i.e. surrounding water, food). While, Merrifield and Rodiles (2015) reviewed that gut morphology, rearing environment, and diet are likely to shape the gut microbiota. In the present study, we detected a relatively higher load of *Streptococcus* spp. and *Aeromonas* spp. with a higher prevalence of *Aspergillus* spp. in the intestine from tilapia from aquacultures while this was not the status in the case of *Pseudomonas* spp. However, the artificial diet supplemented with protease-prebiotic mixtures can increase intestinal width and length (Abd Elnabi *et al*., 2020; Ahmed *et al*., 2020). This is in agreement with the present study where the data showed that aquaculture fish had the highest villi length and width. Noteworthy, the increased villus length performed anaerobic conditions, which support the facultative anaerobe microorganisms like *Streptococcus* spp. and *Aeromonas* spp., as well as the microaerobic *Aspergillus* spp. but does not support the *Pseudomonas* spp., which is strictly aerobic. Moreover, the oxidation of lipids consumes oxygen, which aids in performing anaerobic conditions that also, support the facultative anaerobes and the microaerobes (Friedman *et al*., 2018). The imbalance between the beneficial and opportunistic pathogen results in inflamed intestinal walls and impaired intestinal immunity (Sitja-Bobadilla *et al*., 2016) thus increasing the possibility of infection.

The microbial infections disturb the intestinal goblet cell differentiation and mucus production (Pian *et al*., 2020) indicating the importance of goblet cells and their mucus as a defensive player in fish immunity by separating the epithelium from intestinal content. Concurrently, increases in GCC and its mucus production resist colonization and help in the expulsion of pathogens (Kim and Ho, 2010). In consistence with this, our study showed that GCC was significantly higher in aquaculture groups, which had a higher load of opportunistic bacteria. Moreover, *Aeromonas sobria*-challenged Nile tilapia showed elevated intestinal GCC (Ahmed *et al*., 2020).

The spleen is the only lymph node-like organ in the teleost. It traps and clears foreign particles, and produces antibodies (Roberts, 2012). The spleen of RI and ADI showed mild and diffuse respectively activation of MMCs and diffuse depletion. The spleen tissue of stressed fish shows increases in the number, size, and/or pigment content of macrophages (Ledic-Neto *et al*., 2014). Moreover, the activation of MMCS is a reliable infection indicator (Fournier *et al*. 2001) and a sensitive biomarker for immune activation (Balamurugan *et al*., 2012), thus supporting the hypothesis that MMCs represent the primitive site of adaptive immune system activation in fish (Steinel and Bolnick, 2017).

In the present study, leukocyte counts significantly decreased in fish from aquaculture compared to those from river waters. Our results are similar to Thangam *et al*. (2014); Shin *et al*. (2016); Hertika *et al*. (2021). Neutrophil was the only leukocyte that showed elevation in aquaculture waters. We suggested that this result indicated a beginning of infection, most likely bacterial. Cooperatively, our histopathological results of the activated MMCs in the spleen of fish of these groups supported this suggestion.

In the present study, there was a marked decrease in the phagocytic activity of phagocytes of aquaculture fish. Our results were approved by Guo *et al*. (2022) who found a significant decline in serum lysozyme and complement C3 and C4 after chronic ammonia exposure as well as, a significant downregulation of mRNA levels of toll-like receptors in the spleen and head-kidney, which could impair the phagocytic activity. Noteworthy, the lysozyme, complement, and toll-like receptors are essential components in the phagocytosis process (Rosales and Uribe-Querol, 2017).

IgM is a major biomarker for evaluating the toxic effects of various environmental stressors (Kim and Kang, 2016a). Our results showed decreased values of IgM of fish from aquaculture waters. Furthermore, Qin *et al*. (2017); Yu *et al*. (2020) and Guo *et al*. (2022) found significant decreases in protein and transcriptional levels of the splenic IgM in Megalobrama amblycephala, Pelteobagrus vachellii, and Rhynchocypris lagowski respectively,

exposed to ammonia.

Concerning hematological parameters, in the present study, we found a significant decrease in the values of RBC, Hb, and PCV in fish living in aquaculture waters compared to those from river waters. Our results were consistence with Al-Zahaby *et al*., (2017); Osman *et al*. (2018), and Gao *et al*. (2021). The reduction in RBCs, Hb, and PCV values may be due to high pH (Kim *et al*., 2021), ammonia (Shin *et al*., 2016), and nitrite (Jia *et al*., 2015). Nitrite affects the regulation of ions in fish tissue (Jensen, 2003). While, high concentrations of ammonia cause an increase in pH and ammonia concentration in the blood of the fish that can affect osmoregulation (Lawson, 1995). The proposed result of this toxicant is the haemodilution of blood due to damage and later bleeding in the gills (Heath, 1995) and\or destruction of mature RBCs, and the inhibition of erythrocyte production (Musa *et al*., 2013).

Monitoring changes in the levels of AST and, ALT enzymes in the fish blood consider a good indicator of fish health condition (Kim and Kang, 2016b). AST and ALT are plasma nonfunctional enzymes, normally localized within the cells of the liver, heart, gills, kidneys, muscles, and other organs (Hadi *et al*., 2009). In the present study, the values of ALT, AST, creatinine, and urea reflected the histopathological picture, where we observed significant elevations of these measurements in aquaculture fish over the river water groups. Moreover, Osman *et al*. (2018) and Mohamed *et al*. (2020) recorded similar results.

CONCLUSION

Aquaculture waters had a higher load of opportunistic bacteria and fungi. Ammonia level of BM (0.18) at pH 7.43 and temperature 27.8 °C caused histopathological changes in tilapia gills. Histopathological alterations in tilapia gills besides surrounding microbial loads of water affected the microbiome composition of gills. The artificial diet, directly and indirectly, increased the facultative anaerobes' loads. Aquaculture waters lowered tilapias' immunological parameters, phagocytic activity, and hematological indices but raised the liver and kidney function markers which indicated their tissue's affections. Fish from ADI showed an increase in neutrophil count with the activation of MMCs.

CONFLICT OF INTEREST

The authors have no relevant financial or non-financial interests to disclose.

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