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ORIGINAL ARTICLES

Application of new strategies to reduce suspended solids in zero-exchange system: I. Histological alterations in the gills of Nile tilapia

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ABSTRACT

An 8-week experiment was conducted for evaluating new strategies to reduce suspended solids in zero-exchange system and using Nile tilapia gills alterations as criteria for the evaluation. An experimental design with six treatments and three replicates per treatment was performed. Four heterotrophic-based system (HS) treatments; daily feeding at normal rates without application of fasting and/ or settling strategies (CH), daily feeding at normal rates and application of settling solid strategy (SH), the amount of feed delivered in full amount but on alternate day without settling (FH), the amount of feed delivered in full amount but on alternate day with application of settling strategy (SFH) and two autotrophic-based system (AS) treatments (daily feeding at normal rates without application of fasting and/or settling strategies (CA), the amount of feed delivered in full amount but on alternate day without settling (FH). Fish reared under SH and SFH treatments had fewer and less severe histopathological lesions in gills than controls and fasting treatments. The observed pathologic gill lesions were; partial or complete fusion of some lamellae and damage in the chloride cells. The most severe gill morphological alterations were observed in the fish reared under FA and FH treatment, the observed pathologic gill lesions were; epithelial lifting of secondary lamella, necrosis, fusion of some filaments, filament disorganization, hypertrophy and hyperplasia of the lamellar epithelial cells with partial or complete fusion of some lamellae, rupture of lamellar epithelium, increase of mucus secretion , aneurysm in the primary and secondary lamella, congestion of the blood vessels and damage of chloride cell damage. Our study demonstrate that settling treatments may consider as an effective strategy to reduce the stress imposed on the fish and to protect the aquatic habitats under zero-exchange systems and only a maximum concentration of 220-250 mg SS/1 is recommended as a guideline.

Key words: *Suspended solids, Nile tilapia, zero-exchange, heterotrophic, autotrophic and gill histology.*

Introduction

Beside the conventional water treatment systems, there are other possible techniques which are used to recycle aquaculture water and simultaneously produce fish feed within the pond. These double-purpose techniques are a bacteria based systems technology, which can be used in intensive systems. In addition to the maintenance of good water quality, these techniques provide an inexpensive feed source and a higher efficiency of nutrient conversion to feed (Browdy *et al.*, 2001; Burford *et al.*, 2004; Moss, 1995 and Wasielesky *et al.*, 2006). The bacteria based systems which depend on heterotrophic bacteria can utilize diets lower in protein than more conventional systems because of the extensive grazing by the fish on the bacterial floc and algae in the system. Two kinds of feeding occur in this technique: feeding microorganisms in water in rearing units (feeding the heterotrophic bacteria with pure carbon source) and feeding the fish with low cost fish diets (cheap low protein diet). In addition, this system is very suitable for areas facing a shortage of water sources, since it is operated with much less water than in traditional intensive pond systems. This approach is also called biofloc technology (BFT).

The microorganisms that colonize minimal-exchange, super intensive BFT can include algae, bacteria, and zooplankton. These assemblages are partially contained within bfb oc particles (Hargreaves, 2006 and Wasielesky *et al.*, 2006).

Under BFT total suspended solids (TSS) accumulate in the pond at a fast rate when fish biomass is high. The desired microbial community and reserves of feed are associated with the TSS. However, excessive levels of TSS are not favorable since it can augment biochemical oxygen demand (BOD), species, suppress beneficial algal growth, and promote potentially harmful microorganisms (Beveridge *et al*., 1991; Chapman *et al.*, 1987; Hargreaves, 2006; Brune *et al.*, 2003; Alonso-Rodriguez and Paez-Osuna, 2003; Liltved and Cripps, 1999; Crab

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et al., 2007). In addition, if water mixing is not well controlled, or when TSS concentration exceeds the mixing capacity of the system, solid particles settle down and may accumulate and create anaerobic layer or pockets. The existence of anaerobic sites in the pond bottom may lead to the production of toxic reduced compounds and eventually severely hamper fish growth.

Being the major organ for respiration and osmoregulation (Hughes and Morgan 1973), fish gills are directly exposed to, and affected by, suspended solids in the water (Mallatt 1985, Laurent and Perry 1991, Perry and Laurent 1993). Damage in integrity of gill lamellae is likely to reduce capacity for oxygen transfer and ammonia excretion, and lead to respiratory stress and ammonia intoxication (Randall and Daxboeck 1984, Randall and Wright 1987). Furthermore, it is generally believed that SS plumes caused by dredging and dumping sediments may affect fish, yet few studies have been conducted to measure the response of marine fish to a range of concentrations and exposure durations relevant to dredging and dumping (Wilber and Clark 2001).

Therefore, the objectives of this study were: a) Test new strategies that will reduce the levels of accumulation of suspended solids in tilapia production units under zero-exchange biofloc system. b) Study the effect of TSS level on gill histopathology

Materials and Methods

This study was carried out between 1 June and 26 July 2012 (8 week) in the Fish Nutrition Laboratory (FNL) of the Animal Production Department, Faculty of Agriculture, Cairo University. An experimental design with six treatments and three replicates per treatment was performed. The treatments consisted of:

1- Four heterotrophic- based system (HS) treatments;

- Control treatment under heterotrophic- based system condition (CH): Daily feeding at normal rates without application of fasting and/ or settling strategies.
- Settling strategy under heterotrophic- based system condition (SH): Daily feeding at normal rates and application of settling solid strategy.
- Alernte day fasting strategy under heterotrophic- based system condition (FH): The amount of feed delivered in full amount but on alternate day without settling.
- Settling and fasting heterotrophic strategy (SFH): The amount of feed delivered in full amount but on alternate day with application of settling strategy.

2- Two autotrophic-based system (AS) treatments;

- Control treatment under autotrophic-based system condition (CA): Daily feeding at normal rates without application of fasting and/or settling strategies.
- Alternate day fasting strategy under autotrophic - based system condition (FH): The amount of feed delivered in full amount but on alternate day without settling.

The experimental units were plastic tanks (200 L) provide with constant aeration from a 1 HP blower, through plastic tubing and air diffusers. Three tanks per treatment were employed. The tanks for the AS treatments were exposed continuously to sunlight. The tanks for HS treatments were put under constant shadow and covered with a black mesh layer to minimize the sunlight exposure. In the tanks of the AS treatments the proportion C: N was maintained around 5: 1 using an agricultural fertilizer (N: P / 40: 4). In the tanks of HS, the C: N was maintained around 20: 1, by using molasses (70% of daily feed amount) to provide organic carbon, following the specifications of Avnimelech, (2009)**.** No water exchange was done during the experiment, and only the evaporated water was replaced each three days with well water.

Nile tilapia fry (*Oreochromis niloticus* L.) purchased from a commercial hatchery located in Kafr El-Sheikh Governorate, Egypt. The experimental fish were apparently healthy. They were carefully transferred to the lab and kept for a conditioning period of 14 days. A total number of 360 tilapia fingerlings with an average body weight of 33.35 ± 2.51 g were stocked in the experimental units at a rate of 20 fish/tank (100/m^3). Tilapia fingerlings were additionally fed a commercial diet with 30% of crude protein at a rate of 3% of total biomass per day. Diet was prepared using locally available feed ingredients. Feed supplied was weekly adjusted by weighting tilapia each experimental unit.

Total suspended solids:

Water samples (50 ml) were collected weekly at around 12:00 h from each tank and filtered under vacuum pressure through pre-dried and pre-weighed GF/C filter paper. The filtered water was used for nutrient analysis and the filter paper for total suspended solid (TSS) determination. Total suspended solid (TSS) were measured on a weekly basis following Stirling, (1985). After filtering water for nutrient analysis, the pre-dried and weighed filter paper containing suspended materials was dried in an oven until constant weight. Dried samples were weighed to 0.01 mg. The TSS was calculated from the weight differences.

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Histopathological analysis:

A gill arch of the right side of each fish was sampled and dehydrated in graded ethanol concentrations and embedded in paraffin wax. Sagittal sections were cut and mounted on glass slides. Sections were deparaffinized in xylene, hydrated in ethanol and stained with hematoxylin/eosin. Changes induced by treatment in the gill tissue were photographed under a light microscope (Leica, DM 750) equipped with a Leica camera ICC50 HD and LAS EZ Software.

Results and Discussion

Total suspended solids concentrations and the histological alterations found in the gill of the fish reared under zero-exchange treatments are detailed in Table 1. Fish reared under SH and SFH treatments had fewer and less severe histopathological lesions in gills than controls and fasting treatments. The observed pathologic gill lesions were; partial or complete fusion of some lamellae and damage in the chloride cells (Fig. 2 and 4).

Moderate alterations were observed for the fish reared under CH and CA treatments, gills showed necrosis and fusion in secondary lamellae, hypertrophy in epithelial cells, increase in the mucus secretion, aneurysm in the primary and secondary lamella, chloride cell damage and congestion and constricted blood sinus (Fig. 1and 5).

The most severe gill morphological alterations were observed in the fish reared under FA and FH treatment, the observed pathologic gill lesions were; epithelial lifting of secondary lamella, necrosis, fusion of some filaments, filament disorganization, hypertrophy and hyperplasia of the lamellar epithelial cells with partial or complete fusion of some lamellae, rupture of lamellar epithelium, increase of mucus secretion, aneurysm in the primary and secondary lamella, congestion of the blood vessels and damage of chloride cell damage (Fig. 3 and 6).

These observations disagree with those reported by Azim and Little (2008) who reported that there is no evidence of potential gill damage due to presence of biofloc. The similar observation was made by Vincent (2006) who compared fish raised over extended periods within BFT and RAS systems in a commercial farm. Lake and Hinch (1999) found gills of coho salmon (*Oncorhynchus kisutch* L.) were neither visually affected nor clogged with suspended sediment after exposure to 250 mg SS l^{-1} for 96 h.

Generally, high levels of TSS affect on the aquatic habitats as indicated by poor growth, fusion of gill lamellae (Mettam, 2005) and susceptibility to bacterial or parasite infections (Noble and Summerfelt, 1996). Very little studies is available on gill histopathological of fish exposed to high concentrations of SS for extended periods` (Wilber and Clarke 2001). Acute exposure to high SS concentrations on the gill of salmonid by clogging or coating of gills, causing coughing, respiratory stress and mortality (Hughes and Morgan 1973, Servizi and Gordon 1990, New- combeand MacDonald 1991, Servizi and Martens 1991).

Lifting of lamella epithelium is the most commonly reported lesion in fish gills in the present study which caused edema of lamella and reduced the inter lamellar space, which may impede water flow across the respiratory surface, causing respiratory stress in the fish specially for fish reared in FH and FA tanks. Soivio and Heikrala (1981) showed that fish under hypoxic conditions developed symptoms of epithelium thinning, thickening of pillar cell system as well as a distension of the vasculature in gill lamellae. These responses are essential to facilitate gaseous exchange by shortening the diffusion distance and increasing the residence time for blood at the respiratory surface (Soivio and Heikrala, 1981). Similar gill pathological changes were observed in the fasting treatments, suggesting that the fish were under hypoxic stress.

The chloride cells on the gill filament are the site of sodium and chloride translocation, using Na^+, K^+ - ATPase to create ionic and electrical gradients that are used for salt secretion in seawater (Avella and Bornancin, 1989; McCormick, 1993). Changes in chloride cell num-Au et al.: Chronic effects of suspended solids on fish ber could lead to a corresponding change in Na^+, K^+ - ATPase activity (Lemaire-Gony *et al*. 1994). Our data showed increase of chloride cell density with increasing concentrations of suspended solids in fasting treatments and controls treatments. This may be explained by the fact that the increase in number of chloride cells was able to maintain osmoregulatory processes via Na^+, K^+ -ATPase at lower SS concentrations in settling treatments. However, at a high SS concentration controls and fasting treatments, even an increase in chloride cells was unable to compensate for the respiratory distress experienced by the tilapia.

The study demonstrate that settling treatments had fewer and less severe histopathological lesions in gills and may consider as an effective strategy to reduce the stress imposed on the fish and to protect the aquatic habitats under zero-exchange systems and only a maximum concentration of 220-250 mg SSl^{-1} is recommended as a guideline.

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Table 1: Histopathological alterations in the gill of the fish reared under zero-exchange treatments.

		CH	SH	FH	SFH	CA	FA
	TSS (mg/l)	488	247	280	220	510	318
N	Lesion						
1	Epithelial lifting	-	-	***	-	***	-
2	Necrosis	**	-	***	-	**	***
3	Lamellar fusion	**	**	**	*	***	***
4	Hypertrophy	**	**	**	-	**	-
5	Hyperplasia	-	-	-	-	***	**
6	Epithelial rupture	-	-	**	-	-	-
7	Mucus secretion	**	-	**	-	**	***
8	Aneurism	***	**	***	*	***	***
9	Congestion	**	*	**	-	**	**
10	Mucus cell proliferation	-	-	-	-	-	-
11	Chloride cell damage	**	**	***	*	***	***
12	Chloride cell proliferation	-	-	-	-	-	-
13	Leucocytes infiltration	-	-	-	-	-	-
14A	Dilated blood sinus	-	*	-	-	-	-
14B	Constricted blood sinus	**	-	-	-	-	**

None (-), mild (*), moderate (**) and severe (***)

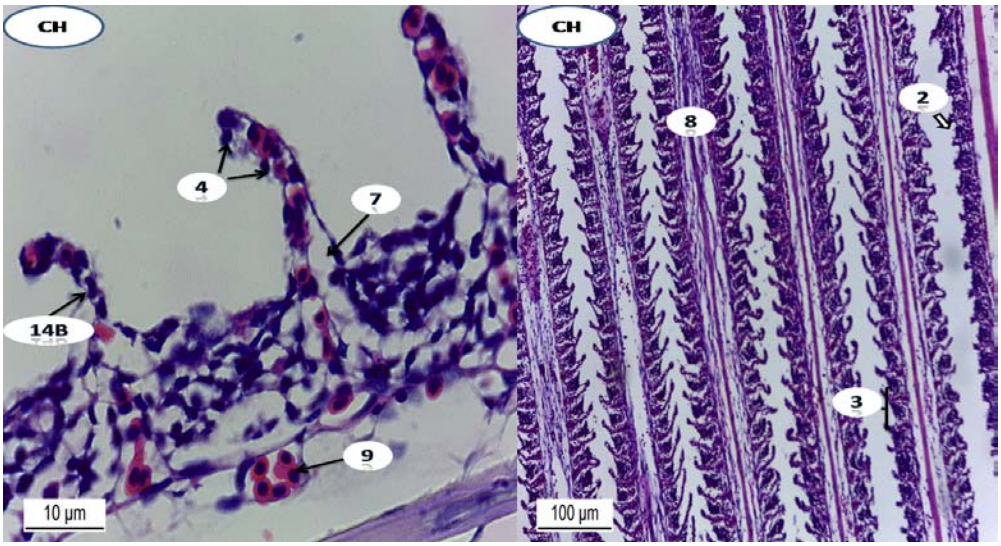

Fig. 1: Gill histological sections of Nile tilapia from (CH) treatment. Necrosis (2), Lamellar Fusion (3), Hypertrophy (4), Mucus Secretion (7), Aneurism (8), Congestion (9) and Constricted Blood Sinus (14B).

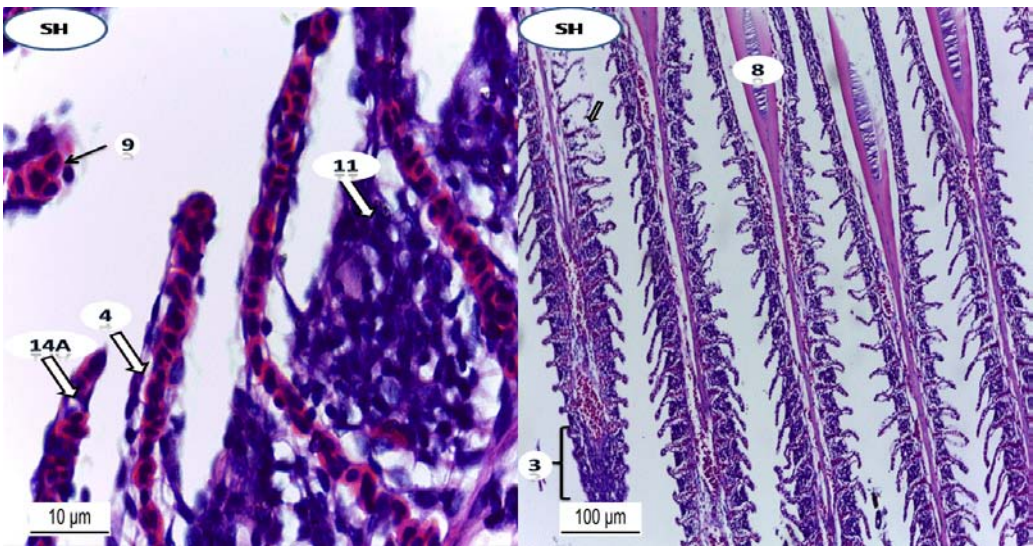

Fig. 2: Gill histological sections of Nile tilapia from (SH)) treatment. Lamellar Fusion (3), Hypertrophy (4), Aneurism (8), Congestion (9), Chloride Cell Damage and Dilated Blood Sinus (14A).

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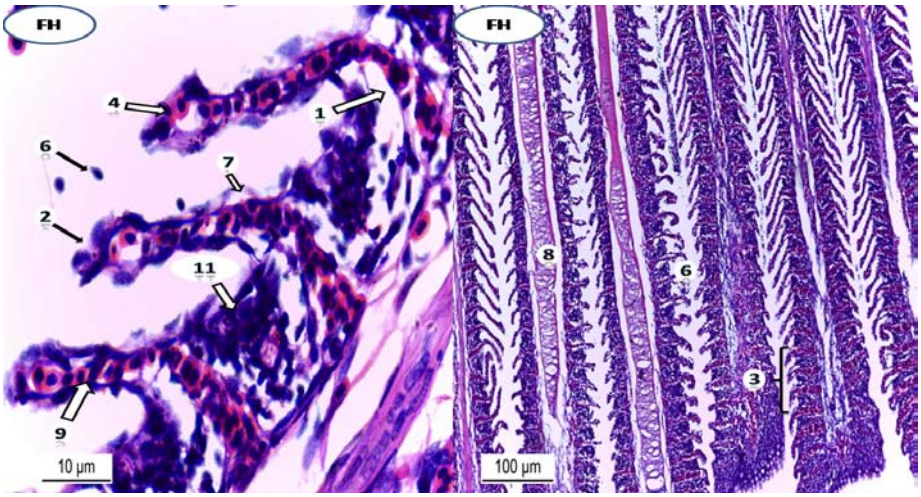

Fig. 3: Gill histological sections of Nile tilapia from (FH)) treatment. Epithelial Lifting (1), Necrosis (2), Lamellar Fusion (3), Hypertrophy (4), Epithelial Rupture (6), Mucus Secretion (7), Aneurism (8), Congestion (9) and Chloride Cell Damage (11).

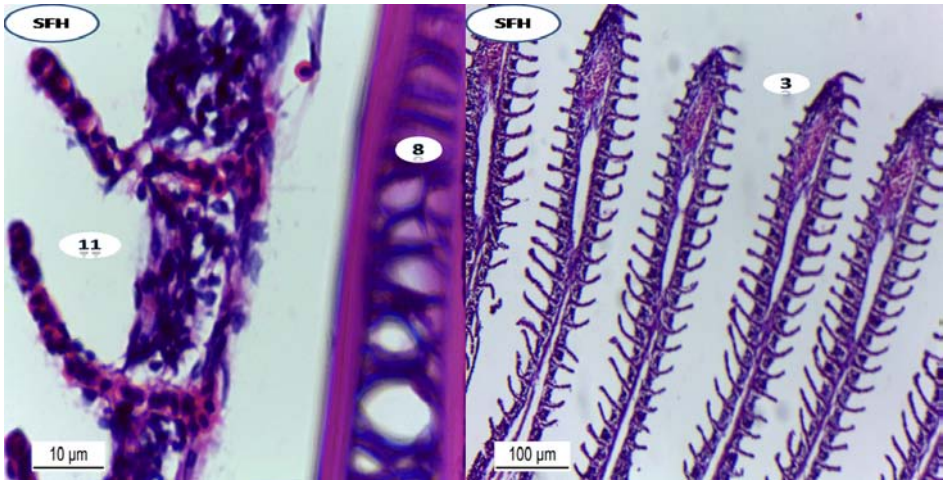

Fig. 4: Gill histological sections of Nile tilapia from (SFH)) treatment. Lamellar Fusion (3), Aneurism (8) and Chloride Cell Damage (11).

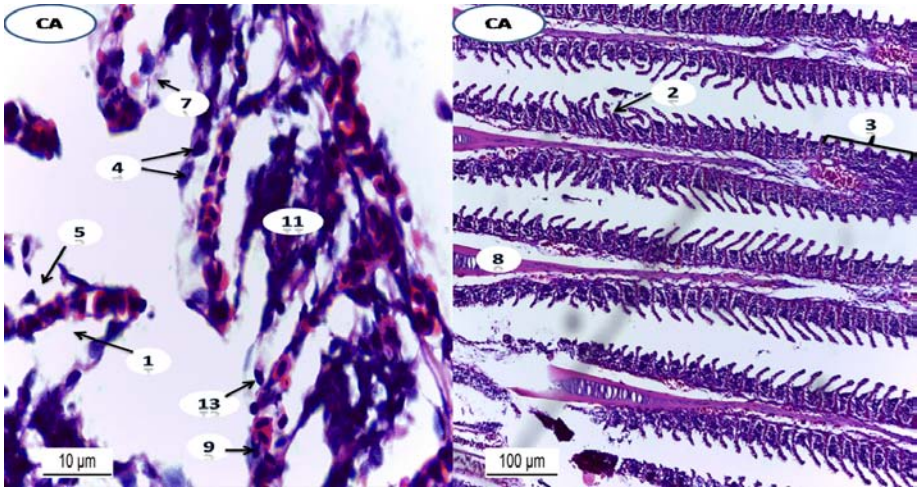

Fig. 5: Gill histological sections of Nile Tilapia from (CA)) treatment. Epithelial Lifting (1), Necrosis (2), Lamellar Fusion (3), Hypertrophy (4), Hyperplasia (5), Mucus Secretion (7), Aneurism (8), Congestion (9) and Chloride Cell Damage (11).

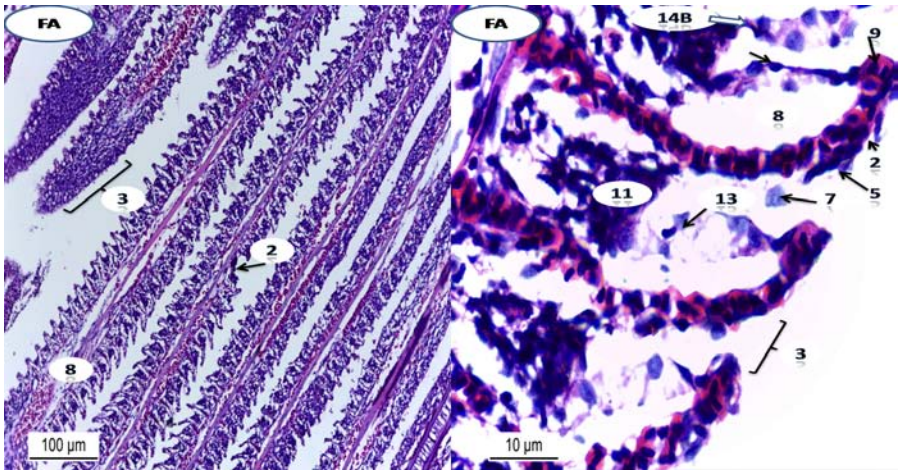

Fig. 6: Gill histological sections of Nile Tilapia from (FA)) treatment. Necrosis (2), Lamellar Fusion (3), Hyperplasia (5), Mucus secretion (7), Aneurism (8), Congestion (9), Chloride Cell Damage (11) and Constricted Blood Sinus (14B).

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