



Efficacy of dietary Biogen® supplementation with water treatment with QZ Toss™ on Nile Tilapia

Moshira Thabet¹, Gaafar Alkhateib², Mahmoud Tanekhy³, Abd elwahab Alsenosy⁴, Abdalla Elbially^{1,*}, Samar Abaz¹, Magdy Soliman¹

1 Department of Poultry and Fish Diseases, Faculty of Veterinary Medicine, Damanhour University, Damanhour 22511, El-Behera, Egypt

2 Hydrobiology department, veterinary research division, national research center, Egypt

3 Department of Poultry and Fish Diseases, Faculty of Veterinary Medicine, Alexandria University, Alexandria, Egypt

4 Department of Biochemistr, Faculty of Veterinary Medicine, Damanhour University, Damanhour 22511, El-Behera, Egypt

ABSTRACT

The current study was designed to investigate the probiotic potential of the Biogen® with and without the water quality improvement product (Q Z Toss™) on growth and health performance of Nile tilapia (*Oreochromis niloticus*). Four fish groups were maintained on control diet supplemented with the Biogen® for one month with or without inclusion of Q Z Toss™ in water. Dietary levels of Biogen® exhibited significant raise in growth parameters with no harmful impacts on hepatic functions. Importantly, Q Z Toss™ supplemented group showed obvious decline of water ammonia level. However, water ammonia level didn't change in Biogen® treated fish compared to control. The histopathological examination revealed better hepatopancreas and intestinal epithelial status in Biogen® treated groups than other groups. Therefore, we can recommend the dietary addition of Biogen® in aqua-feed along with Q Z Toss™ application in rearing water as a proficient method to achieve feasible and sustainable fish health.

Keywords: Biogen®; Q Z Toss™; Biochemical; Histopathological

1. Introduction

Fish represent a valuable source of protein and essential micronutrients that are essential for a balanced diet and good health (Reverter et al., 2014). Fish accounted for 30% of total animal protein in the diet of Asian populations, 20% in Africa and 10% in Latin America (Gayani et al., 1996).

With increasing demand for environment-friendly aquaculture, the use of probiotics and plant extracts in aquaculture is now widely accepted (Wang et al., 2008).

The excessive and unscientific use of antimicrobials in aquaculture led to the development of antimicrobial-resistant microorganisms that can cause hard-to-treat diseases (Das et al., 2013; Soliman et al., 2014). Additionally, using excessive antibiotics without regulation can produce antibiotic residues in the fish body that may be harmful for human consumption (Gupta et al., 2019).

Probiotics have been proposed as an alternative solution for minimizing antimicrobial resistance since it can reduce pathogenic organisms in the gastrointestinal tract of fish through their competitive colonization ability on the host's intestine (Mohammadian et al., 2018)

Biogen® is a commercially available product containing plant extracts and probiotics. among its major components are allicien (the product of

*Corresponding author:

E-mail address: abdallakhiry@gmail.com

Department of Poultry and Fish Diseases, Faculty of Veterinary Medicine, Damanhour University, Damanhour 22511, El-Behera, Egypt

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garlic) + *Bacillus Subtilis* + Ginseng + high unit hydrolytic enzymes (amylolytic, and lipolytic, proteolytic) (Elam, 2004).

Various studies have studied the beneficial effects of using allicin on poultry and fish health (Abdel-Daim et al., 2015; Hussein et al., 2013; Nya et al., 2010).

Q Z Toss™ (Q B lab, Atlanta Georgia, The USA) is a commercially product containing a probiotic bacterial mixture. Q Z Toss™ is a special blend of three probiotic microbial cultures: *Bacillus Subtilis*, *Bacillus Lichenformis*, and *Bacillus Megaterium* (Khan, 2014).

Here, we studied various health, growth and biochemical parameters following the use of Biogen® and, Q Z Toss™. Additionally, we studied whether there is a synergistic effect of combing both Biogen® and, Q Z Toss™ on fish health.

2. Material and methods

2.1. Fish

We used a total number of 180 apparently healthy Nile tilapia *Oreochromis niloticus* (*O. niloticus*) fish with average body weight 50 ± 5 gm in the experimental work. Fish were obtained from a private fish farm in El-Beheira Governorate and transported alive to the experimental facility in aerated plastic tanks.

2.2. Experimental tanks

Throughout the experimental period, fish were kept in 6 prepared concrete tanks ($3 \times 4 \times 1$ m. each).

Prior to the experimental period, fish were acclimatized for 2 weeks. The tanks were supplied with deep well water according to Innes (1966).

The continuous aeration was maintained in each pond using a 3hp electric air pump. Water temperature was kept naturally at 24 ± 1 °C.

2.2.1. Fish diets:

A floating fish pellets containing 30% crude protein (Aller Aqua Egypt) was used for fish feeding throughout the experiment. Amount of feeding was performed according to the fish size, Since the experimental fish body weight is around 50 gm/ fish, feeding was provided at 5% of body weight as described by Eurell et al. (1978). The daily amount of food was offered on two occasions over day (at 9 AM and 1 PM).

2.2.2. Probiotics

1. Biogen® was obtained from the El-Zahra Veterinary Trading (exclusive agent of the manufacturer, China Way Corporation, Taiwan). The Biogen® constants were as follows: Allicin (not less than 0.247 Mmol g^{-1}), *B. subtilis* Natto (not less than 6×10^7 g^{-1}) and High Unit Hydrolytic Enzyme (not less than 3690 U g^{-1}).

2. Q Z Toss™:

As confirmed by the manufacturer (Keeton Industries USA), it's a blend of *Bacillus* species contains 2×10^{12} cfu/kg namely, *Bacillus subtilis* 9×10^{11} cfu, *Bacillus amyloliquefaciens*, 8×10^{11} cfu, and *Bacillus lichenformis*, 3×10^{11} cfu/kg.

2.3. Preparation of experimental feed

Biogen® was mixed with the diet in a ratio of 1000 gm/ton feed with sunflower oil, applied and mixed with the feed and then left for drying.

2.4. Experiment

Table 1: Body weight, blood and serum biochemical parameters

	Control -Ve	Q Z Toss™ only	Biogen® + Q Z Toss™	Biogen® only
Body weight (g)	71.27 ± 0.37	74.33 ± 0.33	80.03 ± 0.06 *	80.80 ± 0.72 *
AST (U / ml)	11.23 ± 0.15	11.19 ± 0.46	9.72 ± 0.83	10.27 ± 0.37
ALT (U / ml)	36.00 ± 1.53	36.33 ± 0.88	36.67 ± 1.20	37.83 ± 2.83
TLCs x 103/mm3	4.80 ± 0.17	4.94 ± 0.12	4.57 ± 0.18	4.75 ± 0.14

Data represented as mean± SE. Within rows values with different superscripts indicating that their corresponding means are significantly different at ($p \leq 0.05$) according to one-way ANOVA followed by Tukey -b test.

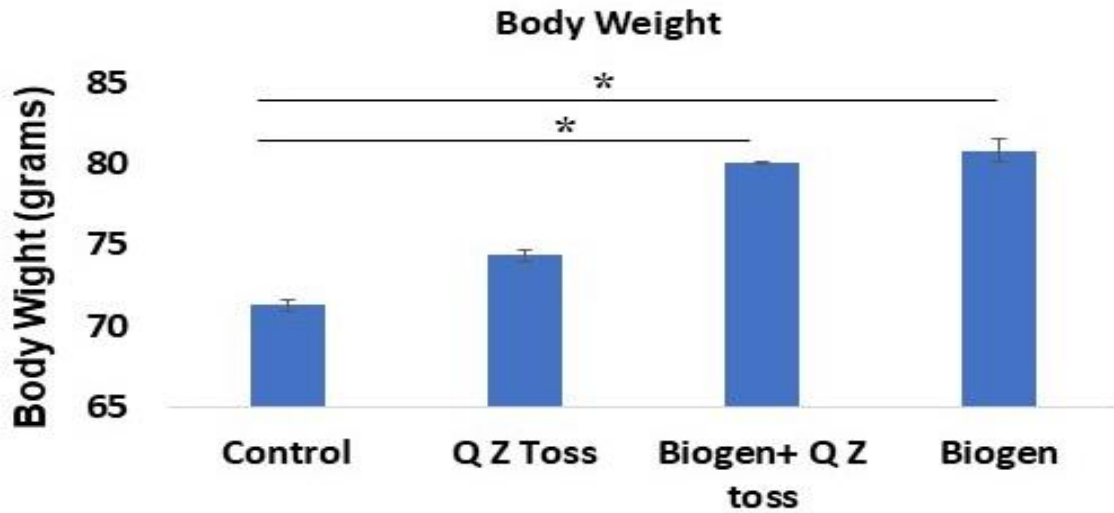


Figure (1): Body weight of Nile Tilapia after treatment with Q Z Toss™ in water and Biogen® at 500mg/kg of food. Statistical differences ($P < 0.05$) are denoted by asterisks. Data are expressed as the mean ± SE (n = 3).

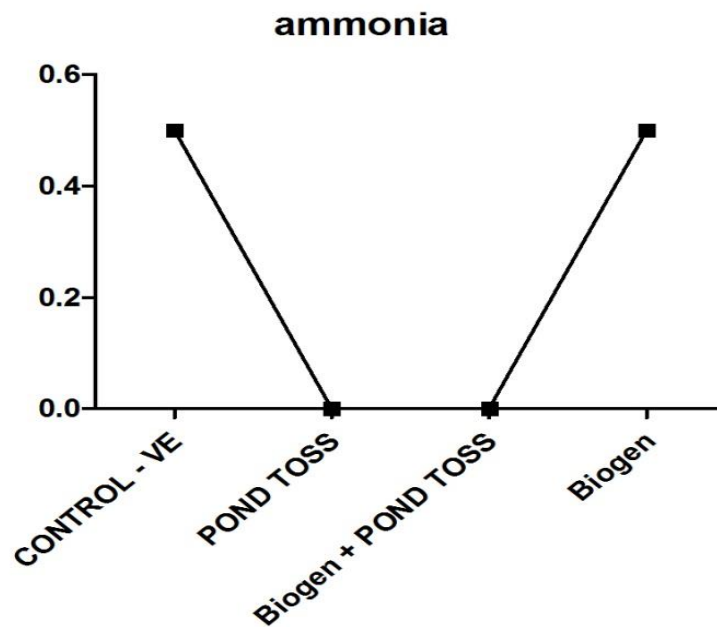


Figure (2): Water levels of ammonia in fish groups.

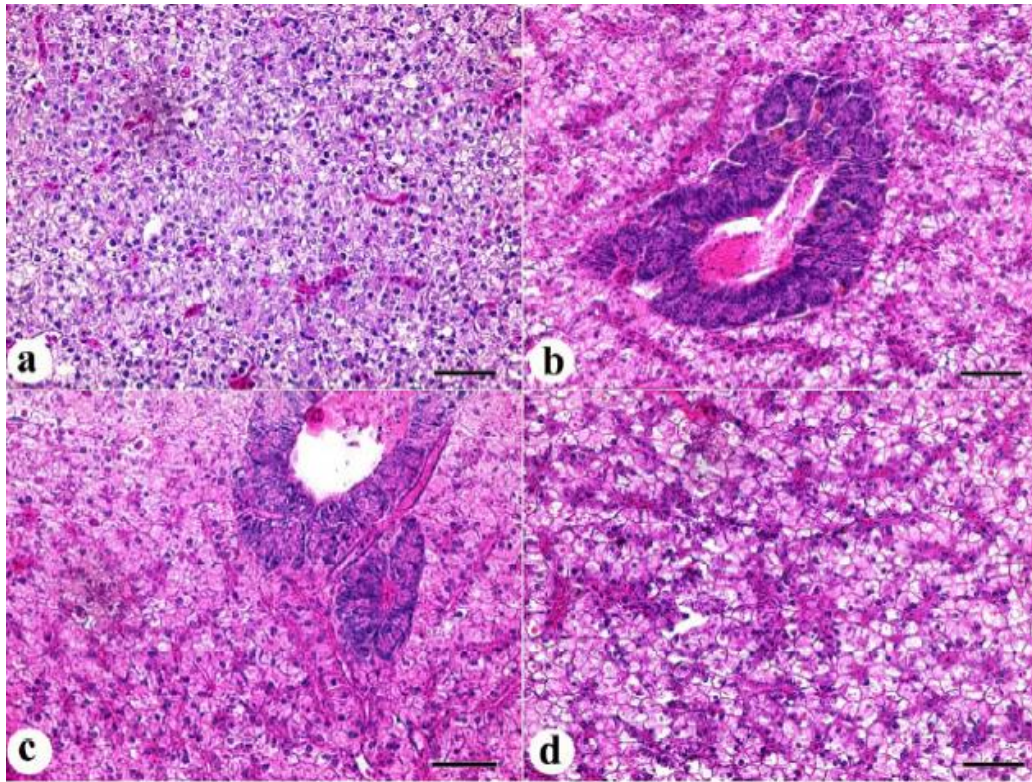


Figure (3): Hepatopancreas of Nile Tilapia (*Oreochromis niloticus*) (a) Control -ve group showing mild congestion in sinusoidal spaces with some vacuolar degeneration in hepatocytes. (b) Q Z Toss™ only group showing mild congestion in main blood vessels and sinusoidal spaces. (c) Biogen® 1 kg/ton + Q Z Toss™ group showing mild congestion in main blood vessels and sinusoidal spaces. (d) Biogen® 1 kg/ton only group showing mild congestion in sinusoidal spaces with some vacuolar degeneration in hepatocytes. Hematoxylin & Eosin stain (Bar = 50 μ m).

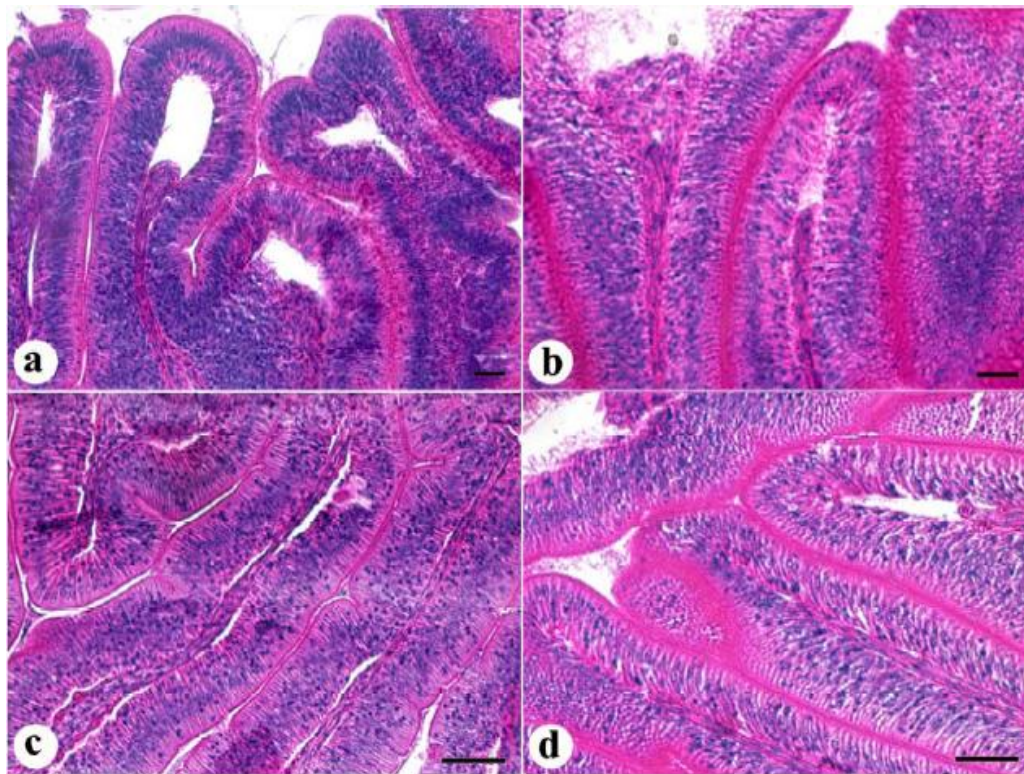


Figure (4): Intestine of Nile Tilapia (*Oreochromis niloticus*) (a) Control -ve group showing moderate sub-epithelial edema. (b) Q Z Toss™ only group showing mild sub-epithelial edema. (c) Biogen® 1 kg/ton + Q Z Toss™ group showing normal healthy villar epithelial structure. (d) Biogen® 1 kg/ton only group showing normal healthy villar epithelial structure. Hematoxylin & Eosin stain (Bar = 50 μ m).

One hundred eighty *O. niloticus* fish were distributed randomly in 4 concrete tanks, 30 fish / tank which filled with aerated deep well water. Fish in the 1st tank were fed basic feed till the end of experiment (30 days) and act as negative control. Fish in 2nd tank were fed basic feed, as well as Q Z Toss™ in a dose of 2gm/ m3 was added to the water, after that Q Z Toss™ was added again as 1gm/m3 each week till the end of experiment. Fish in the 3rd tank were fed on ration containing 500 gm/ ton Biogen® with addition of Q Z Toss™ exactly like the 2nd group. Fish in the 4th tank were fed on ration containing 500 gm/ ton Biogen® without application of Q Z Toss™ till the end of experiment.

The water in tanks receiving Q Z Toss™ remained without change till the end of experiment, while the water in the tanks without Q Z Toss™ was changed daily. The amount of ration was re-adjusted every week according to the fish body weight. Fish were kept under observation for any up normal signs. The level of ammonia in each tank was determined by ammonia kits at the end of experiment. At the end of the experiment the fish were weighted to estimate the growth rate.

2.5. Sampling

2.5.1. Blood samples

At the end of the experiment, the fish was immobilized on absorbent paper towel and kept motionless. The body surface was then cleaned and blotted dry. The blood samples were collected from the caudal vein on EDTA to determine white blood cell count. Other blood samples were collected without anticoagulants for serum separation. The serum samples were stored at -20 °C for biochemical analysis.

2.5.2. Tissue specimens

After complete necropsy of the fish, fresh tissue specimens were collected from hepatopancreas and intestine were rapidly fixed in Davidson's fixative for 24 hours then transferred to 70% ethanol till processing proceeds, for histopathological examination.

2.5.3. Determination of some biochemical parameters

The serum samples were used to measure alanine aminotransferase (ALT) and aspartate aminotransferase (AST), they were determined colorimetrically according to the methods described by Reitman and Frankel (1957), respectively.

2.5.4. Total leucocyte count (TLC)

The total leucocyte count was determined by haemocytometry.

2.5.5. Histopathological examination

The fixed tissue specimens were processed through the conventional paraffin embedding techniques (Suvarna et al., 2018). Paraffin blocks were cut as 4 µm-thick tissue sections. Then 2 replicates from the same section were mounted on slides then processed for hematoxylin-eosin (H&E) staining, cover-slipped then visualized by Light Microscope (Olympus BX43).

2.6. Statistical analysis

GraphPad Prism 5 (San Diego, USA) was used for statistical analysis using one-way Analysis of Variance (ANOVA). ($p < 0.05$) was used for declaration of significance between groups.

3. Results

3.1. Growth performance

The bodyweight of *O. niloticus* fish fed on Biogen® was significantly enhanced ($P \leq 0.05$) compared to the control group (Table 1, Figure 1). Also combining Biogen® with Q Z Toss™ has a significant effect on fish growth performance.

3.2. Ammonia levels in water

Q Z Toss™ supplementation in fish tanks water reduced ammonia levels in water as showed in figure (2). Both Q Z Toss™ supplemented groups (Q Z Toss™ only and Biogen® with Q Z Toss™) showed an apparent decline of water ammonia level as we can see in Figure 2.

3.3. Haematogram and serum parameters

Total leucocyte count (TLC) results showed no significant changes between experimental groups and the control (Table 1). Additionally, Liver enzymes of Biogen® and Q Z Toss™ supplemented groups showed no significant difference for AST, ALT compared to the control group (Table 1). Indicating that there are no harmful effects of using Q Z Toss™ and Biogen® on liver function.

3.4. Histopathological findings

Hepatopancreas of all treated fish showed congestion in main blood vessels and sinusoidal spaces, with some vacuolar degeneration in hepatocytes (Figure 3), in Biogen® + Q Z Toss™ group there was mild activation of melano-macrophage centers (Figure 3c). Intestine of all Biogen® treated fish groups showed normal healthy villar epithelial structure (Figure 4c, d), while in control -ve group and Q Z Toss™ only group mild sub-epithelial edema was observed (Figure 4a,b).

4. Discussion

The excessive and non-regulated use of broad-spectrum chemotherapeutics has led to the spread of drug-resistant microbes (Das et al., 2013; Soliman et al., 2014).

Probiotics and plant extracts can act as substitutes to antimicrobials since they have the ability to reduce pathogenic organisms colonization in the gastrointestinal tract of fish (Mohammadian et al., 2018).

Using probiotics and plant extract are not limited to their antimicrobial effect. An increasing number of studies highlighted the fundamental role of probiotic bacteria on fish health and immunity (Nandi et al., 2017; Perez-Sanchez et al., 2011). Here we studied some of the health effects of using Biogen® together with Q Z Toss™ in Nile Tilapia.

In this study, Dietary supplementation of Biogen® significantly enhanced growth rate in Nile Tilapia. That may be attributed at least in part to the high unit hydrolytic enzymes which are among the components of Biogen®. According to (Yang and Yu, 1990), these enzymes make the starch, fat and protein of feeds to be entirely dissociated and absorbed in gastrointestinal tracts. However, Q Z Toss™ supplementation did not significantly enhance growth rate

The water ammonia level is one of the most important water quality parameters that affect fish health (Bhatnagar and Devi, 2013). Unlike animals that excrete their nitrogenous waste in the form of urea, fish excrete nitrogenous waste in the form of ammonia which is more toxic. that makes periodic elimination of ammonia from fishponds is a necessity in aquaculture (Hargreaves and Tucker, 2004; Ip and Chew, 2010). In addition to the fish body, decomposition of organic matter is a major source of ammonia in the fish water system, therefore, maintaining low ammonia level is crucial for healthy fish growth (Hargreaves and Tucker, 2004).

Here we showed that regardless of Biogen® supplementation, the inclusion of Pond Toss™ in fish tanks significantly reduced ammonia level. these results do make sense because *Bacillus* strains (the main component of Pond Toss™) could convert ammonia to nitrogen under aerobic conditions (Kim et al., 2005).

The present biochemical results didn't show any significant changes in ALT and AST level compared to the control group, indicating that Biogen® treatment at a concentration of 500 gm/ ton is safe and has no harmful effect on liver function tests. and this is an important point to consider.

The histopathological picture is another important aspect for studying the beneficial effects of using probiotics and plant extracts.

The present histopathological examination revealed an obvious improvement of pathology in fish intestine treated with Biogen® with or without the inclusion of Q Z Toss™.

In conclusion, this study revealed no deleterious effects due to supplementation of Nile tilapia feed with commercial probiotic (Biogen®) on liver function with an improvement of the histopathology of the hepatopancreas and intestine. Importantly Biogen® and Q Z Toss™ supplementation significantly enhanced fish growth rate and reduced water ammonia level. Altogether, we can recommend the dietary inclusion of Biogen® in aquafeed along with Q Z Toss™ application in rearing water as an efficient method to achieve feasible and sustainable fish health status.

Competing Interests

The authors have no conflict of interest.

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