Damanhour Journal of Veterinary Sciences 3 (1), (2020) 12-16

Damanhour Journal of Veterinary Sciences

Journal homepage: https://djvs.journals.ekb.eg/

Efficacy of Dietary Probiotic Supplementation with Inclusion of Q Z Toss™ on Nile Tilapia

Ali Abo Esmael [1,*], Mohamed Elgohary [1], Alkhateib Gaafar [2], Mahmoud Tanekhy [3], Abd Elwahab Alsenosy [4], Abd Allah Elbialy [5], Samar Abaza [5], Magdy Soliman [5]

[1]Unit of Fish Diseases, Animal Health Research Institute, Kafr El-Sheikh Branch, Egypt

[2]Department of Hydrobiology, Veterinary Research Division, National Research Centre, Egypt

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

[4]Department of Biochemistry, Faculty of Veterinary Medicine, Damanhour University, Damanhour, Egypt

[5]Department of Poultry and Fish Diseases, Faculty of Veterinary Medicine, Damanhour University, Damanhour, 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. Both Biogen® dietary levels exhibited significant increase in growth parameters as well as in white blood cell count with no negative impacts on renal functions. The histopathological examination revealed better renal epithelial status in Biogen® treated groups than other groups, while gills showed significant improvement due to Q Z Toss™ treatment more than other untreated groups. Therefore, we can recommend the dietary inclusion of Biogen® in aqua-feed along with Q Z Toss™ application in rearing water as an efficient method to achieve feasible and sustainable fish production.

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

1. Introduction

Aquaculture, however, is an increasingly important option in animal protein production. This activity requires high-quality feeds with a high protein content, which should contain not only the necessary nutrients but also complementary additives to keep organisms healthy and promote favourable growth. Some of the most utilized growth-promoting additives include hormones, antibiotics, ionophores and some salts (lara-flores et al., 2003).

The aquaculture ability to reduce the resultant exhaustion of wild fisheries and enhance economic development has brought the industry to be the most dynamic food sector. The fish farming intensification puts fish under risk of infectious diseases (Snieszko 2006). One of the solutions to improve the animal health is the use of functional dietary supplements (Ganguly 2013).

The reduction of pH level in the stomach and upper intestine increase the number of the intestinal beneficial flora such as lactic acid producing bacteria (Abu Elala & Ragaa 2015) and inhibit the growth of Gram-negative bacteria through the dissociation of the acids (SCFAs) and production of anions in bacterial cells (Hoseinifar et al., 2017; Nawaz et al., 2018).

*Corresponding author:
E-mail address: Vet_aboesmael@yahoo.com
1Unit of Fish Diseases, Animal Health Research Institute, Kafr El-Sheikh Branch, Egypt
P ISSN: 2636-3003 EISSN: 2636-2996

Received: September 20, 2019; Received in revised form: October 7, 2019; accepted: October 9, 2019.

Due to the negative effects of chemicals and antibiotics on the environment, followed by the development of mutagenic microbial strains and adversely affected fish health, their application to control disease outbreaks is no longer recommended (Cabello 2006). Therefore, the application of eco-friendly feed additives, such as microbial supplements, to improve the physiology, growth performance, and immune responses of aquaculture-related species have gained much more attention during recent years (Dawood and Koshio 2016). Naturally-occurring microorganisms play a key role in aquatic environments, as they can fulfil a wide range of roles, including recycling nutrients, degrading organic matter, and protecting fish against infections (Bentzon-Tilia et al., 2016). All these roles conduced to use these microorganisms in aquaculture and the development of probiotics. The use of probiotics is one of the alternative approaches to immunoprophylactic control in aquaculture (Esteban et al., 2014).

Q Z Toss™ is a probiotic that help improve the water quality on fish and shrimp farms by reducing ammonia and nitrites and by digesting organic matter in the sludge.

Probiotic Biogen® consists of *Bacillus licheniformis* and *Bacillus subtilis*. The advantage of these spore-forming bacteria is that they are able to survive the pelletization process. After transit through the stomach, they germinate in the intestine and use a large number of sugars for their growth and produce a range of relevant digestive enzymes. The beneficial effects of probiotics include higher growth and feed efficiency, prevention of intestinal disorders and pre-digestion of anti-nutritional factors present in the ingredients (El-Haroun et al., 2006). Hence, probiotics in aqua feed should be tested for their efficacy as has already been demonstrated in animal production systems. Many studies on probiotics in aquaculture have used *in vitro* models of specific bacteria as antagonists of pathogens (Vine et al., 2006).

The current study was conducted to assess the efficacy of Biogen® on health of cultured Nile tilapia along with addition of Q Z Toss™ to enhance the water quality.

2. Material and methods

2.1. Fish

A total number of 180 apparently healthy Nile tilapia *Oreochromus niloticus* (*O. niloticus*) fish with average body weight 50±5 gm were used 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

Fish were kept in 4 prepared concrete tanks (3X4X1 m. each). These tanks were used for holding the experimental fish throughout the acclimatization and experimental period of this study.

Table 1: Body weight, WBCs and serum biochemical parameters

	Control -Ve	**Q Z Toss™**	**Biogen + Q Z toss**	**Biogen**
Body weight (g)	71.27 ± 0.37	74.33 ± 0.33	80.03 ± 0.06 *	80.80 ± 0.72 *
Urea (mg/ dl)	3.58 ± 0.07	3.97 ± 0.26	3.75 ± 0.14	3.78 ± 0.15
Creatinine (mg/dl)	0.51 ± 0.02	0.56 ± 0.01	0.46 ± 0.02	0.42 ± 0.04
TLCs x 10^3/mm^3	4.80 ± 0.17	4.94 ± 0.12	4.57 ± 0.18	4.75 ± 0.14

Data represented as means± 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*.

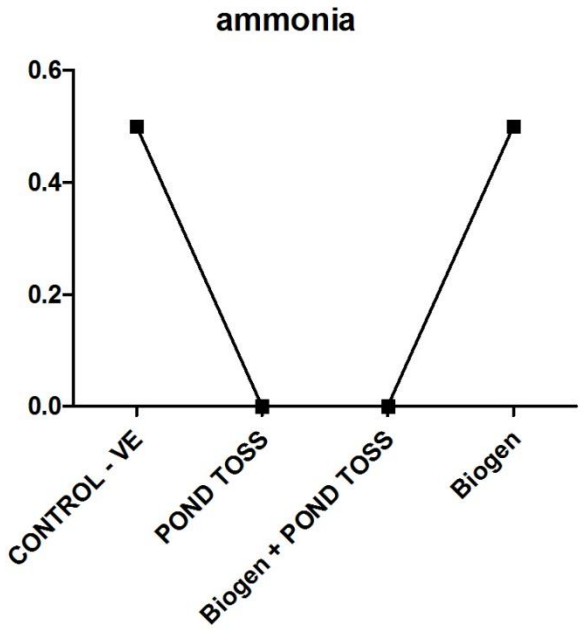

Figure 1: Water levels of ammonia in fish groups

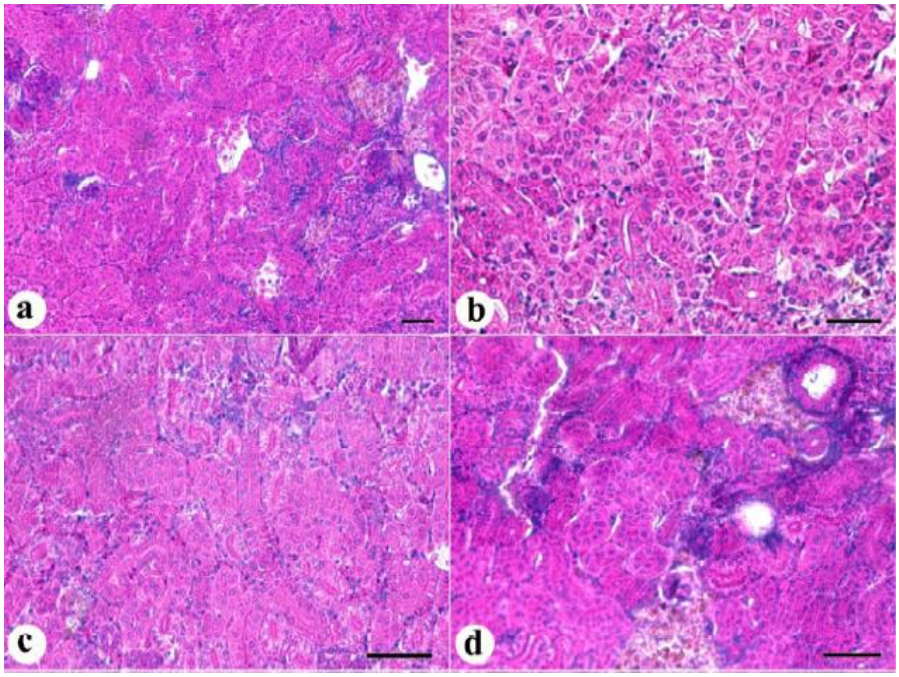

Figure 2: Posterior kidney of Nile Tilapia (*Oreochromis niloticus*) (a) Control –ve group showing focal areas of renal tubular necrosis. (b) Q Z toss only group showing normal tubular structure. (c) Biogen 1 kg/ton + Q Z toss group showing normal tubular structure. (d) Biogen 1 kg/ton only group showing normal tubular structure with activation of melano-macrophage centers. Hematoxylin & Eosin stain (Bar = 50 µm).

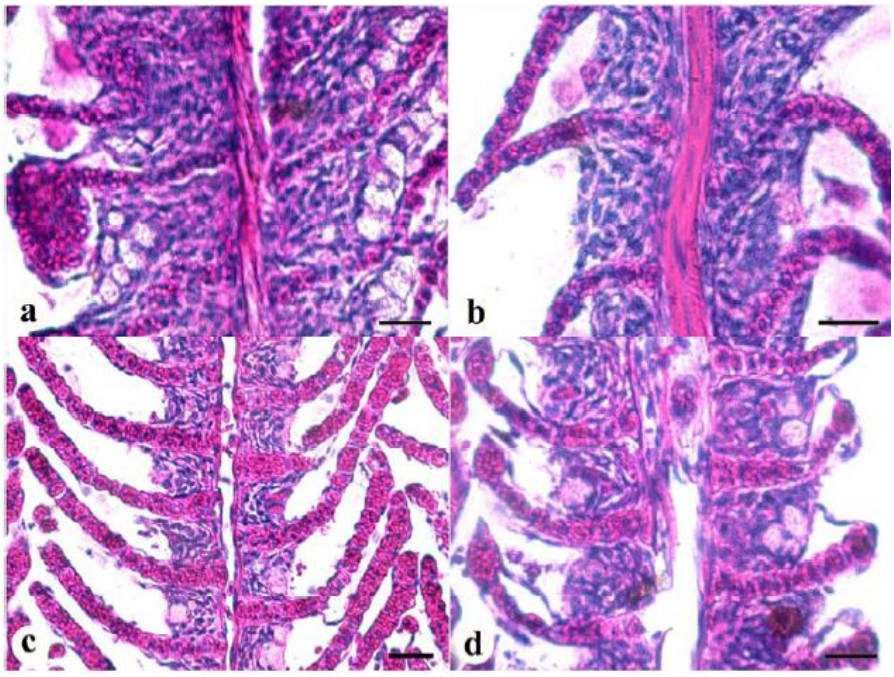

Figure 3: Gills of Nile Tilapia (*Oreochromis niloticus*) (a) Control –ve group showing severe hyperplasia in malpegian cell layers with pronounced telangiectasis. (b) Q Z toss only group showing moderate hyperplasia in malpegian cell layers. (c) Biogen 1 kg/ton + Q Z toss group showing mild hyperplasia in malpegian cell layers. (d) Biogen 1 kg/ton only group showing moderate hyperplasia in malpegian cell layers. Hematoxylin & Eosin stain (Bar = 50 µm).

All fish were acclimatized for 2 weeks prior to the experiment. The tanks were supplied with deep well water according to (Behrens et al., 2017) The continuous aeration was maintained in each Q Z using a 3hp electric air pump. Water temperature was kept naturally at 24±1 ºC.

2.3. Fish diets

Fish were fed floating fish pellets containing 30% crude protein (Aller Aqua Egypt). According to the used fish size, the diet was daily 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).

2.4. Probiotics

1. Biogen®

It 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 confirmd 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*, $8X10^{11}$ cfu, and *Bacillus lichinformis*, 3×10^{11} cfu/kg..

2.5. Preparation of experimental feed

The diets were prepared by mixing the Biogen® in a ratio of 1000 gm/ton feed with sunflower oil, applied and mixed with the feed and then left for drying.

2.6. Experiment

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 regular feed till the end of experiment (30 days) and act as negative control. Fish in 2nd tank were fed regular feed, as well as Q Z Toss™ in a dose of 2gm/ m^3 was added to the water, after that Q Z Toss™ was added again as 1gm/m^3 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 and FCR.

2.7. Growth parameters

The nutritional performance in terms of feed intake (FI) (g), initial weight (g), final weight (FW) (g), weight gain (WG) (g), feed conversion ratio (FCR), specific growth rate (SGR) and protein efficiency ratio (Ruyet *et al.*) were calculated bi-weekly for two months according to (Abu Elala & Ragaa 2015).

2.8. Sampling

2.8.1.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 haemoglobin and 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.

2.8.2. Tissue specimens

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

2.9. Determination of some biochemical parameters

The serum samples were used to measure serum urea and creatinine, they were determined colorimetrically according to the methods described by (Fawcett & Scott 1960) and (Bartels *et al.* 1972), respectively.

2.10. Total leucocyte count (TLC)

The total leucocyte count was determined by haemocytometry.

2.11. Histopathological examination

The fixed tissue specimens were processed through the conventional paraffin embedding techniques (Layton and Suvarna 2013). 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).

2.12. Statistical analysis

All data were statistically analysed using one-way Analysis of Variance (ANOVA) using GraphPad Prism 5 (San Diego, USA). All declarations of significance depended on ($p < 0.05$).

3. Results

3.1. Growth performance

The growth performance of *O. niloticus* fish fed on biogen is summarized in Table (1). The results revealed that both Biogen supplemented groups showed increase in live body weight gain which was apparently significant in Biogen® with Q Z Toss™ group ($P \leq 0.05$) compared to control one.

3.2. Ammonia levels in water

Inclusion of Q Z Toss™ in fish tanks water decreased ammonia levels in water as showed in figure (Mart *et al.*), where both groups of Q Z Toss™ (Q Z Toss™ only and Biogen® with Q Z Toss™) showed zero levels of ammonia in water compared to a mean level of (0.5) ammonia in groups without Q Z Toss™.

3.3. Haematogram and serum parameters

The blood picture of fish group fed biogen revealed higher TLC count. All experimental fish groups have normal haematological and serum biochemical findings (Table 1). Where, renal function tests represented in urea and creatinine showed normal values as control for treated groups, excluding any drawbacks of Biogen® supplementation on kidneys function.

3.4. Histopathological findings:

Posterior kidney of all treated fish showed multifocal tubular degeneration and necrosis except Q Z Toss™ only group showing normal tubular structure (Fig. 2), while in Biogen® + Q Z Toss™ group there was activation of melano-macrophage centers (Figure 2c). Gills of all treated fish showed milder degrees of pathology in Q Z Toss™ treated groups represented by moderate hyperplasia in malpegian cell layers (Fig 3b,c), while control –ve group and Biogen® only groups showed severe hyperplasia in malpeghian cell layers with separation of the epithelial lining of the secondary gill lamellae and severe telangiectasis (Fig. 3a, d).

4. Discussion

Dietary supplementation of the Biogen® improved the weight gain and in Nile tilapia. Previous results showed that, probiotics as Biogen improved weight gain, specific growth rate (SGR) and feed efficiency ratio (FER) and hence the survival rate when added to basal diet of *P. clarkii* juveniles. The improvement in growth parameters was due to the change in *Bacillus* bacteria ratio in the gut flora. Significant increase in specific growth rate (SGR) and feed efficiency ratio (FER) of *M. rosenbergii* were detected after the administration of Biogen (Saad et al., 2009). Moreover, using different types of probiotics increased the growth performance of Nile tilapia, rabbit fish and European perch (El-Haroun et al., 2006). On the other hand, administration of 3% Biogen to basal diet of *P. clarkii* juveniles had no effect on weight gain, SGR and FER. These results agreed with the study of (Mona et al., 2015). Positive effects of Biogen administration in the daily diet may be due to the *Bacillus* bacteria found in Biogen that secrete a wide range of exoenzymes and the presence of probiotics might stimulate the production of endogenous enzymes by the freshwater fish (Wang 2007). Furthermore, it may be due to the effect of the tested probiotics which improved absorption of nutrients and depressed harmful bacteria (Ghazalah et al., 2010).

The positive impact of Biogen® on haematological and serum biochemical parameters was evidenced by normal white blood cell count, normal renal function tests, and their histopathological picture. Dietary administration of Biogen improved immune response of *P. clarkii*juveniles due to an increase in phagocytic activity of granulocytes under the effect of *Bacillus* (Belkaid and Hand 2014). Also, probiotics may induce a higher haemocyte proliferation (Rodríguez et al., 2007) and it may be due to change in the microbial community of the specimen which led to better immune responses (Li et al., 2007). This could be attributed to potential non-specific responses in fish. Better renal tissue status in histopathology and activation of melanomacrophage centres in biogen group were as a result of macrophage activation due to Biogen®.

Maintaining good water quality is important in aquaculture as the quality of water affects the health and growth of the fish. Good water quality can also help improve the Feed Conversion Ratio which in turn improves fish size and profits. Ammonia concentrations are regulated by passive diffusion down the partial pressure gradient of NH_3 across the gill (Wilson et al., 1994). This gradient is maintained by the simultaneous active excretion of protons from the gills. At the gill surface, protons bind to ammonia molecules, resulting in the formation of ammonium ions.

Excess levels of total ammonia present a major obstacle to intensive fish culture, as high volumes of uneaten feed and fecal matter lead to the accumulation of nitrogenous waste (Suzer et al., 2008). Unmanaged total ammonia concentrations in aquaculture are known to compromise fish health, retard growth and cause mortality (Ruyet et al., 1997).

The present study revealed that concentrations of ammonia were observed to be low in treated Q Zs than in the control Q Z. Probiotics are instrumental in maintaining good water quality, higher beneficial and lower pathogenic bacteria loads in fish Q Zs (Qin et al., 2016).

In conclusion, this study revealed no deleterious effects due to supplementation of Nile tilapia feed with commercial probiotic (Biogen®) on the function and integrity of gills and posterior kidney with an improvement in water quality via Q Z Toss. Altogether, we can recommend the dietary inclusion of Biogen® in aqua-feed along with Q Z Toss™ application in rearing water as an efficient method to achieve feasible and sustainable fish production.

Competing Interests

The authors have no conflict of interest.

References

Abu Elala, N.M., Ragaa N.M. 2015. Eubiotic effect of a dietary acidifier (potassium diformate) on the health status of cultured *Oreochromis niloticus*. J. Adv. Res. 6, 621-9.

Bartels, H., Böhmer, M., Heierli, C. 1972. [Serum creatinine determination without protein precipitation]. *Clin. Chim. Acta;* international J. clinical chemistry 37, 193-7.

Behrens, J.W., van Deurs, M., Christensen, E.A.F. 2017. Evaluating dispersal potential of an invasive fish by the use of aerobic scope and osmoregulation capacity. PloS one 12, e0176038.

Belkaid Y., Hand T.W. 2014. Role of the microbiota in immunity and inflammation. Cell 157, 121-41.

Bentzon-Tilia, M., Sonnenschein E.C., Gram L. 2016. Monitoring and managing microbes in aquaculture-Towards a sustainable industry. Microb. Biotechnol. 9, 576-84.

Cabello, F.C. 2006. Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. Environ. Microbiol. 8, 1137-44.

Dawood, M.A.O., Koshio, S. 2016. Recent advances in the role of probiotics and prebiotics in carp aquaculture: A review. Aquaculture 454, 243-51.

El-Haroun, E., Goda, A., Chowdhury, M.A.K. 2006. Effect of dietary probiotic Biogen ™ supplementation as a growth promoter on growth performance and feed utilization of Nile tilapia *Oreochromis niloticus* (L.). Aquacul. Res. 37, 1473-80.

Esteban, M.A., Cordero, H., Martinez-Tome, M., Jimenez-Monreal, A.M., Bakhrouf A., Mahdhi A. 2014. Effect of dietary supplementation of probiotics and palm fruits extracts on the antioxidant enzyme gene expression in the mucosae of gilthead seabream (*Sparus aurata L*.). Fish Shellfish Immunol. 39, 532-40.

Eurell, T.E., Lewis, D.H., Grumbles, L.C. 1978. Comparison of selected diagnostic tests for detection of motile *Aeromonas septicemia* in fish. Am. J. Vet. Res. 39, 1384-6.

Fawcett. J.K., Scott J.E. 1960. A rapid and precise method for the determination of urea. J. Clin. Pathol. 13, 156-9.

Ganguly, S. 2013. Potential non-antibiotic growth promoting dietary supplements for animal nutrition: A Review. J. Appl. Pharmaceut. Sci. 3, 174-8.

Ghazalah, A., Ali, H., Gehad, E., Hammouda, Y., Abo-State, H. 2010. Effect of Probiotics on performance and nutrients digestibility of Nile tilapia (*Oreochromis niloticus*) Fed Low Protein Diets. Nature and Science 8.

Hoseinifar, S.H., Sun Y.-Z., Caipang, C.M. 2017. Short-chain fatty acids as feed supplements for sustainable aquaculture: an updated view. Aquaculture Res. 48, 1380-91.

Lara-flores, M., Olvera-Novoa, M., Guzmán-Méndez, B.z., López-Madrid, W. 2003 Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus*, and the yeast *Saccharomyces cerevisiae* as growth promoters in Nile Tilapia (*Oreochromis niloticus*). Aquaculture 216, 193-201.

Layton, C., Suvarna K. 2013. Bancroft's Theory and Practise of Histological Techniques (7th edition) (Co-author).

Li, P., Burr, G., Gatlin, D., Hume, M., Patnaik, S., Castille, F., Lawrence, A. 2007. Dietary Supplementation of Short-Chain Fructooligosaccharides Influences Gastrointestinal Microbiota Composition and Immunity Characteristics of Pacific White Shrimp, *Litopenaeus vannamei*, Cultured in a Recirculating System. J. Nutrit. 137, 2763-8.

Martínez Cruz, P., Ibáñez, A.L., Monroy Hermosillo, O.A., Ramírez Saad, H.C. 2012. Use of Probiotics in Aquaculture. ISRN Microbiol. 2012, 13.

Mona, M., Rizk, E., Salama, M., Lotfy, M. 2015. Efficacy of probiotics, prebiotics, and immunostimulant on growth performance and immunological parameters of *Procambarus clarkii* juveniles. J. Basic Appl. Zool. 69, 17-25.

Nawaz, A., Bakhsh, Javaid, A., Irshad, S., Hoseinifar, S.H., Xiong, H. 2018. The functionality of prebiotics as immunostimulant: Evidences from trials on terrestrial and aquatic animals. Fish Shellfish Immunol. 76, 272-8.

Qin, Y., Hou, J., Deng, M., Liu, Q., Wu, C., Ji, Y., He, X. 2016. Bacterial abundance and diversity in pond water supplied with different feeds. Sci. Reports 6, 35232-.

Rodríguez, J., Espinosa, Y., Echeverría, F., Cárdenas, G., Román, R., Stern, S. 2007. Exposure to probiotics and β-1,3/1,6-glucans in larviculture modifies the immune response of *Penaeus vannamei* juveniles and both the survival to white spot syndrome virus challenge and pond culture. Aquaculture 273, 405-15.

Ruyet, J.P.-L., Galland, R., Le Roux, A., Chartois, H. 1997 Chronic ammonia toxicity in juvenile turbot (*Scophthalmus maximus*). Aquaculture 154, 155-71.

Saad, A., Habashy, M., Sharshar, K. 2009. Growth Response of the Freshwater Prawn, *Macrobrachium rosenbergii* (De Man), to Diets Having Different Levels of Biogen. World Appl.Sci. J. 6.

Snieszko, S. 2006. The Effects of Environmental Stress on Outbreaks of Infectious Diseases in Fishes. J.Fish Biol. 6, 197-208.

Suzer, C., Çoban, D., Kamaci, H., Saka, Ş., Fırat, K., Otgucuoğlu, Ö., Küçüksari, H. 2008. *Lactobacillus* spp. bacteria as probiotics in gilthead sea bream (*Sparus aurata*, L.) larvae. Aquaculture 280, 140-5.

Vine, N.G., Leukes, W.D., Kaiser, H. 2006. Probiotics in marine larviculture. FEMS Microbiol. Rev.30, 404-27.

Wang, Y.-B. 2007. Effect of probiotic on growth performance and digestive enzyme activity of shrimp *Penaeus vannamei.* Aquaculture 269, 259-64.

Wilson, R., Wright, P., Munger, S.T.E.V.E., Wood, C., 1994. Ammonia excretion in freshwater rainbow trout (*Oncorhynchus mykiss*) and the importance of gill boundary layer acidification: lack of evidence for Na+/NH4+ exchange. J. Exp. Biol. 191(1), 37-58.