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Mohamed H Bahnasawy
Zoology Department, Faculty of Science, Damietta University, Egypt

Ahmed E El-Ghobashy
Zoology Department, Faculty of Science, Damietta University, Egypt

El-Sayed H El-Ebiary
Fish Rearing Lab., Aquaculture Division, National Institute of Oceanography and Fisheries, Alexandria, Egypt

Amr M Helal
Fish Rearing Lab., Aquaculture Division, National Institute of Oceanography and Fisheries, Alexandria, Egypt

Doaa M El-Sisy
Fish Rearing Lab., Aquaculture Division, National Institute of Oceanography and Fisheries, Alexandria, Egypt

Corresponding Author:
Mohamed H Bahnasawy
Zoology Department, Faculty of Science, Damietta University, Egypt

Effect of probiotic on water quality, growth performance and body composition of nile tilapia (*Oreochromis niloticus*)

Mohamed H Bahnasawy, Ahmed E El-Ghobashy, El-Sayed H El-Ebiary, Amr M Helal and Doaa M El-Sisy

Abstract
The present study was implemented to evaluate the effect of probiotic on improving water quality, growth performance and body composition of Nile tilapia. However, probiotic (EM.1®) was added to rearing water at levels of (0.0 ppm, 50 ppm, 100 ppm, 200 ppm, 300 ppm, 400 ppm and 500 ppm). Water analysis indicated that probiotic application at level of 200 ppm in rearing water significantly improved Dissolved Oxygen (9.02±0.48 mg/l), while it decreased ionized ammonia (0.77±0.03 mg/l) and Un-ionized ammonia (0.04±0.01 mg/l). The maximum fish growth and the best food conversion ratio (1.49±0.07) were obtained at level of 200 ppm. Chemical composition of whole-body fish was significantly affected by probiotic adding to rearing water. The best protein content (13.85±0.21) was obtained at level of 200 ppm. Thus, the present study recommends using probiotic (EM.1®) in rearing water with level of 200 ppm to improve water quality and to enhance fish productivity.

Keywords: Probiotic, water quality, nile tilapia, growth performance

Introduction

During the last decades, chemical additives and veterinary medicines, especially antimicrobial agents have been also applied in aquaculture to prevent and control disease (Lupin, 2009) [32]. Previous studies also show the aquatic bacteria can develop resistance genes as a consequence of exposure to antimicrobial agents (Kim *et al*., 2004; Sorum, 2006) [28, 51]. Therefore, the need for alternative techniques is increasing and the contribution of probiotics may be considerable. Thus, the use of probiotics in aquaculture is now widely accepted with an increasing demand for environment friendly aquaculture (Wang, 2007; Qi *et al.*, 2009) [58, 43]. Nowadays, a number of preparations of probiotics are commercially available and have been introduced to fish, shellfish and molluscan farming as feed additives, or are incorporated in pond water (Prado *et al.*, 2010) [42].

The commercial probiotics EM.1® which contains a mixture of about 80 species of beneficial naturally occurring microorganisms including mainly lactic acid bacteria, photosynthetic bacteria, yeasts and actinomycetes. All of these are mutually compatible and proved to have a reviving action on humans, animals and the natural environment (Xu, 2000) [59].

The Nile tilapia, *Oreochromis niloticus* is widely cultured in many tropical and subtropical countries of the world (Authman *et al.*, 2009) [7], which grew 12% annually, from less than a half million tons (383,654 mt) in the early 1990s to over 5 million tons in the mid-2010s (FAO, 2017).

Therefore, the present study was carried out to evaluate the addition of probiotic (EM.1®) to the rearing water and to evaluate its effect on water quality parameters, growth performance, and the meat quality of Nile tilapia.

Materials and Methods

Nile tilapia (*O. niloticus*) fries (0.10±0.015 g with mean total length of 1.82 cm) were stocked into 100-L aquaria at a density of 200 fry per aquarium. Fish were frequently fed a practical diet (45% crude protein) at a rate of 15% of fish live body weight twice a day for 6 weeks.

The EM.1® probiotic was added to aquaria water at levels of 0.0 (control), 50, 100, 200, 300, 400, and 500 ppm in triplicates.

Fish of each aquarium were samples every week for recording length and weight data. Evaluation of growth performance and feed utilization efficiency including weight gain per cent WG (g), average daily gain ADG (g), specific growth rate SGR, feed conversion ratio FCR, protein efficiency ratio PER, survival rate (SR%) and condition factor (K) were calculated according to Bahnasawy *et al.*, (2003) [3]. Protein, fat, moisture and ash contents were all determined by standard methods of Association of Official Analytical Chemist (AOAC, 1995) [5].

Water quality parameters

The water samples were collected once a week from each aquarium to monitor the different physicochemical parameters of water according to standard methods described in APHA (1999) [6]. Water temperature was measured using centigrade thermometer. The pH value was measured pocket digital pH-meter (HANNA instrument, research Model 211 Digital pH meter. Salinity was determined using Thermo, Orion 150A+ Advanced conductivity meter. Dissolved oxygen (DO) and biochemical oxygen demand (BOD) were determined using Winkler's method and were calculated as mg/l, according to the methods described by (Grasshoff, 1999) [26]. The ionized ammonia (NH_4^+), un-ionized ammonia (NH_3), nitrite (NO_2), and nitrate (NO_3) were determined using a spectrophotometer (Prim Advanced V9b S/N 2667).

Statistical analysis

The obtained data were subjected to analysis of variance (one-way ANOVA) to evaluate the effect of probiotic application. Differences between means were tested at the 5% probability level using Duncan test. All the statistical analyses were done using SPSS program ver. 16 (SPSS, Richmond, VA, USA) as described by Dytham (2011) [20].

Results

The various water quality parameters during the experimental period were recorded as shown in Table 1. The mean water temperature recorded in this experiment was 27.4±0.3 °C. The maximum mean value of DO (9.02±0.48 mg/l) was obtained with fish treated with 200 ppm of EM.1®. Significant differences were found between the mean values of DO in the different treatments. (P>0.05). Concerning the maximum mean value of BOD (*5.43*±0.28 mg/l) that was recorded at control. While, the minimum mean value was recorded in 500 ppm of EM.1® (4.39±0.29 mg/l). A significant difference was detected between groups, (P≤0.05). The maximum mean value of pH (8.25±0.02) was recorded with the control, whilst the minimum mean value of pH (8.11±0.04) was recorded with 500 ppm of EM.1®. The pH mean value at 500 ppm was varied significantly from control, 50 ppm and 100 ppm (P≤0.05).

NH_4^+ results revealed that the minimum mean value recorded with 500 ppm of EM.1® (0.62±0.05 mg/l) compared with control (1.02±0.19 mg/l). There are no significant differences between 200 ppm, 300 ppm, 400 ppm and 500 ppm mean values. (P>0.05). The minimum NH_3 mean value (0.04±0.01 mg/l) had been recorded with 200 ppm of EM.1® compared with the control (0.11±0.02 mg/l). Control, 50 ppm and 100 ppm varied significantly from 200 ppm, 300 ppm, 400 ppm and 500 ppm. (P≤0.05). NH_4^+ mean values are in a reverse pattern with EM.1® concentrations.

The minimum mean value (1.12±0.31 mg/l) was obtained with 500 ppm of EM.1®. Significant decrease in nitrite level is paralleled with a corresponding increase in nitrate Table (1). Moreover it is clear that the maximum mean value of nitrite level has been observed (4.74±0.61 mg/dl) at 500 ppm of EM.1®, compared with control (2.62±0.41 mg/l).

Table (2) shows the growth performance parameters and revealed that the maximum weight gain mean value (2.02±0.15 gm) has been recorded at 200 ppm of EM.1®. The mean weight gain value of 200 ppm was significantly differing from 400 ppm and 500 ppm (P≤0.05). Average daily weight gain (ADG) maximum mean value (0.04±0.0gm) was obtained for 200 ppm of EM.1®. The minimum mean value of FCR (1.49±0.07) had been recorded for 200 ppm. FCR of different EM.1® concentrations were significantly differ from control (P≤0.05). The maximum mean (PER) value (2.24 ± 0.15) was observed with 200 ppm of EM.1®. The maximum mean (SGR) value (6.07±0.15) was obtained also for 200 ppm The maximum SR mean value (94.75±0.7%) was recorded for 200 ppm of EM.1®. Significant differences were found between control and all treated groups. Highly significant correlation was found between EM.1® concentrations and survival rate. The maximum condition factor (K) mean value (2.27±0.02) was obtained for 50 ppm and the minimum mean value (2.01±0.03) was for 400 ppm. No significant differences in K were found in between 500 ppm and the remaining experimental groups.

The whole body composition analysis including moisture, lipid, ash and crude protein are presented in Table (3). The maximum protein content mean value was observed at 400 ppm treatment (14.21±0.45%). No significant differences were found between 200 ppm, 300 ppm, 400 ppm and 500 ppm of EM.1®. (P >0.05). The maximum lipid content mean value (6.36±0.55%) was found with 200 ppm. The moisture minimum mean value (76.24±0.57%) was observed for 500 ppm. No significant differences in moisture content were found between 200 ppm, 300 ppm, 400 ppm and 500 ppm. (P> 0.05). 100 ppm had the maximum Ash content mean value (4.74±0.03%) while the minimum mean value (3.07±0.23%) was for 200 ppm. Control, 50 ppm, 100 ppm were significantly differ from 200 ppm, 400 ppm and 500 ppm (P≤ 0.05). Lipid and protein were correlated positively with the EM.1® concentrations. While, moisture and ash content were reversely correlated with the EM.1® concentrations.

Table 1: Water quality parameters of aquaria stocked with Nile tilapia fries (*Oreochromis niloticus*) and treated with EM.1® probiotic.

	Parameters							
Conc.	**Temp °C Mean ± SE**	**DO mg/l Mean ± SE**	**BOD mg/l Mean ± SE**	**pH Mean ± SE**	**NH_4^+ mg/l Mean ± SE**	**NH_3 mg/l Mean ± SE**	**NO_2 mg/l Mean ± SE**	**NO_3 mg/l Mean ± SE**
Control	27.5±0.12 ^{a}	4.69±0.29 ^{a}	5.43±0.28^a	8.25±0.02^a	1.02±0.19^a	0.11±0.02^a	2.96±0.55^a	2.62±0.41^a
50 ppm	27.7±0.20 ^{a}	5.26±0.41^a	5.23±0.19^a	8.24±0.01^a	0.98±0.04^a	0.10±0.00^a	2.79±0.48^a	2.71±0.48^a
100 ppm	27.3±0.09 ^{a}	5.90±0.21^b	4.79±0.28^{ab}	8.23±0.02^a	0.99±0.05^a	0.10±0.01^a	2.75±0.53^a	2.95±0.47^a
200 ppm	27.4±0.08 ^{a}	9.02±0.48 ^{c}	4.29±0.32^b	8.20±0.04^{ab}	0.77±0.03^b	0.04±0.01^b	1.36±0.38^b	4.01±0.74^b
300 ppm	27.1±0.18 ^{a}	8.98±1.34^c	4.35±0.23^{bc}	8.21±0.04^{ab}	0.70±0.04^b	0.05±0.01^b	1.30±0.38^b	4.12±0.75^b

400 ppm	27.3±0.20^{a}	8.08±1.23^{c}	4.39±0.24^{bc}	8.17±0.07^{ab}	0.63±0.06^{b}	0.06±0.02^{b}	1.23±0.33^{b}	4.71±0.62^{b}
500 ppm	27.4±0.15^{a}	8.83±1.76^{c}	4.39±0.29^{bc}	8.11±0.04^{b}	0.62±0.05^{b}	0.07±0.02^{b}	1.12±0.31^{b}	4.74±0.61^{b}

Different letters represent significant difference ($P<0.05$) within each row of data. No. of replications = 3.

Table 2: Growth performance parameters, survival rate and condition factor of aquaria stocked with Nile tilapia fries (*Oreochromis niloticus*) and treated with EM.1® probiotic.

	Parameters							
Conc.	**WF(g) Mean ± SE**	**WG (g) Mean ± SE**	**ADG (g/day) Mean ± SE**	**FCR Mean ± SE**	**PER Mean ± SE**	**SGR (%) Mean ± SE**	**Survival (%) Mean ± SE**	**K Mean ± SE**
Control	1.08±0.02^{a}	0.98±0.25^{a}	0.019±0.0^{a}	2.11±0.07^{a}	1.58±0.07^{a}	4.16±0.04^{a}	73.75±0.7^{a}	2.06±0.14^{a}
50 ppm	1.48±0.01^{a}	1.38±0.05^{a}	0.027±0.0^{a}	1.76±0.04^{b}	1.90±0.05^{b}	4.48±0.20^{b}	77.00±1.0^{b}	2.27±0.02
100 ppm	1.46±1.02^{a}	1.36±0.19^{a}	0.027±0.0^{a}	1.74±0.01^{b}	1.92±0.02^{b}	4.90±0.15^{b}	80.75±1.2^{c}	2.19±0.00
200 ppm	2.12±0.02^{b}	2.02±0.15^{b}	0.040±0.0^{b}	1.49±0.07^{c}	2.24±0.15^{c}	6.07±0.15^{c}	94.75±0.7^{d}	2.18±0.00
300 ppm	2.05±1.03^{b}	1.95±0.20^{b}	0.039±0.0^{b}	1.55±0.07^{c}	2.16±0.13^{bc}	6.00±0.20^{c}	94.5±0.50^{d}	2.20±0.01
400 ppm	1.30±1.05^{a}	1.20±0.13^{a}	0.024±0.0^{a}	1.72±0.14^{ab}	1.96±0.22^{ab}	5.34±0.06^{d}	94.0±0.50^{d}	2.01±0.03
500 ppm	1.57±1.02^{a}	1.47±0.09^{a}	0.029±0.0^{a}	1.71±0.04^{ab}	1.96±0.06^{ab}	5.47±0.12^{d}	93.5±1.50^{d}	2.11±0.01

Letters represents no significance (P>0.05). No. of replications = 3.

Table 3: Body composition of aquaria stocked with Nile tilapia fries (*Oreochromis niloticus*) and treated with EM.1® probiotic:

	Parameters			
Conc.	**Moisture% Mean ± SE**	**Lipid% Mean ± SE**	**Ash% Mean ± SE**	**Protein% Mean ± SE**
Control	78.96±0.22^{a}	4.51±0.04^{a}	4.51±0.24^{a}	12.02±0.50^{a}
50 ppm	78.37±0.02^{a}	4.68±0.02^{a}	4.62±0.11^{a}	12.32±0.14^{a}
100 ppm	77.78±0.18^{a}	4.86±0.01^{a}	4.74±0.03^{a}	12.62±0.22^{a}
200 ppm	76.72±0.53^{b}	6.36±0.55^{b}	3.07±0.23^{b}	13.85±0.21^{b}
300 ppm	76.97±1.12^{b}	5.35±0.48^{a}	4.62±0.05^{a}	13.06±0.60^{a}
400 ppm	76.74±0.70^{b}	5.74±0.41^{b}	3.31±0.16^{b}	14.21±0.45^{b}
500 ppm	76.24±0.57^{b}	6.21±0.21^{b}	3.49±0.10^{b}	14.06±0.35^{b}

Letters represents no significance (P>0.05). No. of replications = 3.

Discussion

The current results provide clear evidence that probiotics has a great effect to improve water quality of aquaria compared with control. Similar results were recorded by Sunitha and Padmavathi, 2013) [52]. In the present study, the dissolved oxygen was clearly more influenced by the probiotic treatment. Mean BOD values were decreased with the increase of EM.1® concentrations. The present results in agreement with (Zahira, 2015; Wahid and Azman, 2016) [60, 57]. On the other hand, Sahu *et al.*, (2008) [47] have a different opinion that the application of probiotics may be temporarily increased the BOD levels.

Table (1) illustrated that the mean pH values ranged between 8.09 and 8.25, this is within the tolerance range for tilapia (Chervinski, 1982) [17]. Low pH values increases the toxicity of nitrite to cultured organisms (Akpoilih *et al.*, 2015) [2] and high pH increases the un-ionized ammonia (Martinez-Cordova *et al.*, 2011) [34]. Most importantly, however, the present pH values do not significantly differ between the probiotic treatment aquaria and the control, except for 500 ppm this indicating that EM.1® does not significantly affect the pH values.

In general, ammonia exists in water in ionized and un-ionized forms, the both forms levels are balanced depending on the pH and water temperature (Timmons *et al.*, 2002) [52]. NH_3 is the most dangerous on the cultured organisms (El-Sayed, 2006) [22]. In the present study it is evident that the probiotic EM.1® have a conspicuous role in decreasing both the NH_4^+ and NH_3, this may be due to the presence of nitrifying bacteria among EM.1® components which convert ammonia to nitrite and then to nitrate. In addition, actinomycetes have a strong ability to decompose organic matter and fix nitrogen refer to increase their number; this finding was agreed with Chou *et al.*, (2002) [18]. *Lactobacillus* probiotics are another component of EM.1® which is able to remove pathogenic bacteria and improve water quality beside their role in facilitating ammonia elimination from culture water (Ma *et al.*, 2009) [33]. On the other hand Zahira, (2015) [60] mentioned that phototrophic bacterial activity in the EM.1® solution can decompose ammonia. Generally, probiotics as observed in many studies when applied as water additives reduces significantly the ammonia levels this is agreed with (Mohideen *et al.*, 2010; Zokaeifar *et al.*, 2014) [37, 61].

Nitrite considered an intermediate product of nitrification, where nitrification is a process of ammonia conversion to nitrate by two groups of bacteria, i.e. ammonia-oxidizing bacteria which oxidize ammonia to the highly toxic NO_2 and nitrite-oxidizing bacteria which oxidize nitrite to the much less toxic NO_3 as confirmed by (Qi *et al.*, 2009; Ray *et al.*, 2010) [43, 44].

The present results revealed that adding probiotics in fish rearing water is more effective to minimize the toxic effect of nitrogenous compounds (NH_4^+, NH_3 and NO_2) by converting it into less toxic form (NO_3). Similar findings were obtained by **(**Zokaeifar *et al.*, 2014; Sonia *et al.*, 2015) [61, 50].

The best WG%; ADG; FCR and SGR were obtained in the tanks treated with 200 ppm of EM.1®. Since the first use of probiotics in aquaculture, an increasing number of studies have explained their adequacy to get better growth rate of cultured aquatic animals (Lara-Flores *et al.*, 2003; Wang, 2007) [30, 58]. Increasing the growth rate of the tilapia, *O. niloticus*, one of the most important farmed species for the world, was as a result of the rearing water quality increment with probiotics. This was agreed with Bogut *et al.*, (1999) [15] and Noh *et al.*, (1994) [40] for carp with the probiotic *Streptococcus faecium*. A similar finding was also obtained by (Silva *et al.*, 2012) [49].

Improved growth performance of Nile tilapia fed diets with *B. subtilis*, *L. plantarum*, a mixture of *B. subtillis* and *L. plantarum*, and *S. cerevisiae* have been reported by Essa *et* *al.*, (2010) [23]. When Flounder received the *Bacillus* mixture in two separate methods, either by mixing with food or by adding it directly in water. Application of *B. subtilis* and *B. licheniformis* resulted in significant improvement of rainbow trout fry feed conversion ratio (FCR), specific growth rate (SGR), weight gain (WG) and protein efficiency ratio (PER) (Bagheri *et al.*, 2008) [8]. Barnes *et al.*, (2006a) [12] and Barnes *et al.*, (2006b) [13] observed significant improvements in growth and survival rate of Rainbow trout, *Oncorhynchus mykiss* when their diets were incorporated with *S. cerevisiae*-based fermented yeast during the first months of feeding period.

The growth performance parameters in the present experiment were better in all EM.1® treated groups than control. Many authors reinforced the idea on the capacity of probiotics can be improve weight gain (WG), feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER) (Thiam *et al.*, 2015 [53] Munirasu *et al.*, 2017 and Allameh *et al.*, 2017) [38, 4].

Adding EM.1® probiotics in rearing water might be established as an additional source of protein and acted as appetizer where EM.1® consists of a combination of beneficial microorganisms including lactic acid bacteria, *Bacillus sp.* and yeasts and other components this combination had a beneficial effect on growth performance. These results agree with those of Mehrim, (2001) [35] and Diab *et al.*, (2002) [19] for tilapia. The ability of yeast to secrete polyamines such as putrescine, spermidine and spermine have been linked as essential growth factors as reported by Tovar-Ramı´reza *et al.*, (2002) [55] for *S. boulardii*, *S. cerevisiae* and *D. hansenii* respectively. In addition to this, Bardócz, (1993) [11] reported that the polyamines play an essential role in proliferation, rapid growth and regeneration of tissues.

Competitive exclusion of probiotics could be the key factor known for improving the microbial intestinal balance. This possible mode of action which leads the replacement of beneficial bacteria in the gastro intestinal tract might subsequently contribute the growth performance (Balcazar *et al.*, 2006; Zokaeifar *et al.*, 2012) [10, 62].

Studies have demonstrated that probiotics can help control pathogens and increase the welfare and survival rates of reared fish larvae (Mohammadi *et al.*, 2015 & Khatun and Saha, 2017) [36, 27]. In the present study, the higher survival rate increased by adding probiotics in water; this may be due to the administration of significant changes in the proportion of the population of the gut micro flora. This may be exerted by the elimination of harmful bacteria due to establishment of the beneficial microorganisms in the intestine, or colonization of beneficial bacteria may be dominant over harmful bacteria. By their large presence, saturate the adhesion receptors and prevent the pathogenic bacteria from attachment and colonization therefore the present results in agreement with Vine *et al.*, (2004); Seenivasan *et al.*, (2012) [56, 48].

Fulton’s condition factor is an essential parameter used to assess the general health of populations. Low values indicate poor conditions, while high values indicate good conditions (Binohlan and Pauly, 1998) [14].

The proximate chemical analysis of the whole fish body at the end of the experimental period indicated that moisture content was decreased significantly in fish of some treated groups compared with control. This was agreed with Geraylou *et al.*, (2013) [25] Munirasu *et al.*, (2017) [38]. The recent results revealed that adding EM.1® with some concentrations increased the fish lipid content significantly. The same results was recorded by Allameh *et al.*, (2017) [4] who observed an improvement in the lipid content of Nile tilapia when corporate probiotics with diet. This was agreed with Krishnaveni *et al.*, (2013); Munirasu *et al.*, (2017) [29, 38].

From the previous results it was clear that ash content was decreased significantly in some probiotic treated groups compared with control. The same result was detected by Munirasu *et al.*, (2017) [38]. The effect of probiotic on ash content was discussed by Lunger *et al*. (2006) [31] who stated that the ash content of cobia (*Rachycentron canadum*) decreased significantly by dietary probiotic. Contrary, Olvera-Novoa *et al*., (2002) [41] found insignificant decrease in ash content of *O. mossambicus* fed 25 and 35% probiotic.

From the present results it is evident that adding probiotics in rearing water have an improving effect on body composition of Nile tilapia fries. The results indicated that protein content was increased with probiotic treated groups compared with control. Lara-Flores *et al*., (2003) [30] Abdel-Tawwab *et al.*, (2008) [1] stated that the use of *Streptococcus faecium* and *Lactobacillus acidophilus* or *Saccharomyces cerevisiae* improved the protein and lipid content of Nile tilapia. This was agreed with (Reda & Selim, 2015; Allameh *et al.*, 2017) [45, 4]. Moreover, Niang, (2013) [39] Thiam *et al.*, (2015) [53] mentioned that administration of EM.1® probiotic increased the protein content of fish fries than control. Changes in protein and lipids contents of fish body might be due to the presence of probiotics in water which enter with feed to the digestive tract and improved digestibility and utilization of nutrients, in parallel to changes in synthesis and deposition rate of proteins and lipids in fish muscles; this was agreed with (Abdel-Tawwab *et al.*, 2008; Geraylou *et al.*, 2013) [1, 25].

The inverse relationship between fat and water content has been reported by many authors (EL-Ghobashy, 1990) [21] and agreement with the present results. Furthermore, Moisture and ash content decreased while protein and fat levels increased. This is agrees with Munirasu *et al.*, (2017) [38].

Conclusion

The overall results of the present study indicated that the application of probiotics as a water additives is more effective in reducing the levels of nitrite, nitrate. The growth performance parameters were better in all EM.1® treated groups than control and adding probiotics in rearing water have an improving effect on body composition of Nile tilapia fries.

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