THE POSITIVE CONTRIBUTIONS OF PROBIOTIC SUPPLEMENTATION AND ARTIFICIAL SUBSTRATE TO GROWTH PERFORMANCE, FEED UTILIZATION OF NILE TILAPIA.

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SUMMARY

This study is designed to investigate the combined effect of two kinds of commercial probiotic with or without artificial substrate in a periphyton based system on growth performance, feed utilization and chemical body composition on Nile tilapia (*Oreochromis niloticus*). Twelve thousands nine hundred and sixty (12960) Nile tilapia fingerlings with initial weight (1.86g) were used for 105 days. The experiment was carried out as factorial arrangement (2x3) experimental treatments. Two levels of additional periphyton substrate area ($S^{0\%}$, $S^{66.67\%}$) of tank surface were tested where the superscripts refer to artificial substrate levels of 0%, 66.67% of tank area (each of 24 m^3) and three supplementation levels of probiotic (0% without supplementation of probiotic (pro0) and two different commercial probiotic each one supplemented to the basal diet with the recommended level mentioned by the producer 0.1g/kg for the first (Ecobiol Aqua plus®) (pro1) ,2g/kg for the second (Biogen®) (pro2). Each treatment has three replicate of concrete tanks representing 18 concrete tanks (24 m^3each, 8m length x3m width x 1m depth). 720 fingerlings were reared in each concrete tank; fish fed basal diet twice a day. The optimum growth performance and feed utilization of Nile tilapia fingerlings were obtained at artificial substrate $S^{66.67\%}$ in periphyton based system with the second probiotic (Biogen®) suggesting that periphyton should be a part of the diet of fish at commercial and applicable scale.

Keywords: *Periphyton, probiotic, growth performance, feed utilization, Nile tilapia*

INTRODUCTION

There is an urgent need to explore sustainable farming methods in different fish culture to increase fish productivity concerning environmental challenges (Anand *et al*., 2015) .Therefore, Adding substrates in fish ponds, can increase the production of fish when compared with systems without substrate (Keshavanath *et al*., 2001; Van Dam *et al*., 2002; Amisah *et al*., 2008).

Periphyton is an eco-friendly approach in aquaculture, its complex of micro algae heterotrophic bacteria, benthic organisms and detritus developed over submerged substrate in aquatic systems (Azim *et al* ., 2005). This natural food is grazed directly by many omnivorous and herbivorous fish as a basic source of food (Azim *et al*., 2001) , it is practiced successfully in fin fish like Major carp (Keshavanath *et al*., 2004; Keshavanath and Gangadhar 2005; Wahab *et al*., 1999a,b) Tilapias (Hem and Avit 1994; Huchette and Beveridge 2003; Huchette *et al*., 2000; Milstein *et al*., 2005,2009) ; giant fresh water Prawn (Asaduzzaman *et al*., 2010); Penaeid shrimp (Audelo-Naranjo *et al*., 2011; Anand *et al*., 2013) Penaus monodon (Anand *et al*., 2015) polyculture of Tilapia and Prawn (Hasan *et al*., 2012; Rezoanul *et al*., 2016).

Periphyton based system increasing fish production , enhancing nutrient utilization (Abo-Taleb *et al*., 2014) reducing feed costs (Saker *et al*., 2015) improve water quality (Gonzalez *et al*., 2012) ; reduce negative effects of overcrowding (Arnold *et al*., 2009) . These effects were influenced by many factors such as quality and quantity of periphyton, the feeding habits of the species cultured, stocking density, seasonality, substrate type, availability of non periphyton food sources (Azim *et al*., 2003a) and supplementary feed and additives in fish diets reared in this system. From these additives, the probiotic which defined as "a live microbial adjunct which has a beneficial effect on the host by modifying the host associated or ambient microbial community, by ensuring improved use of the feed or enhancing its

nutritional value, by enhancing the host response towards disease, or by improving the quality of its ambient environment" Verschuere *et al*. (2000).

There are many beneficial effects have been reported for probiotics in aquaculture to provide beneficial effects (Blacazar *et al*., 2006)

The positive effect of probiotic administration to fish growth and immune response are well documented (Hussein *et al*., 2016) probiotics can improve growth performance as growth promoter (Ringo *et al*., 2012), induce immune response (Cruz *et al*., 2012), increase resistance against invade pathogenic and survival rate of aquatic animals (Ringo *et al*., 2015).

Nevertheless, the single effects of periphyton area on fish productivity are well known, its combined action with probiotic supplementation in the diet is still poorly understood. So, the present work aimed to determine the effect of probiotic supplementation on growth performance, feed utilization of Nile tilapia (*Oreochromis niloticus*) with or without artificial substrate in periphyton-based system.

MATERIALS AND METHODS

Fish and culture facilities

All-male Nile tilapia, *Oreochromis niloticus*, fingerlings were obtained from commercial tilapia hatchery, Kafr El-Sheikh Governorate, Egypt. Fish were stocked in the reception tanks for two weeks to acclimatize them to the concrete tank conditions. All over the acclimatization period, fish were fed a commercial diet to apparent satiation twice a day (0800 and 1300 h). Twelve thousands nine hundred and sixty (12960) fingerlings with initial weight (1.86 g) were distributed in eighteen concrete tank system each 24 m^3 (8 x 3 x 1 m, length x width x depth) respectively. At a density of 30 fish /m^2 for 105 days. The experimental tanks had a nylon nets (0.5 mm mesh) to prevent fish escape or entry of wild fish. The slurry (sludge) accumulated at the bottoms of the rearing tanks was periodically eradicated by the common siphon method. An artificial aeration was provided to the tanks by two ring blower of 1 kW (1.340 HP), Spencer Vortex® produced by Spencer Turbine Co., Japan. Convex polyethylene greenhouses covered tanks to preserve temperature in the heated water and to absorb solar radiation for heating. The daily water exchange rate used in the ponds was initially about 10%. Daily water exchange was lowered to 1% in all periphyton tanks while the rest nine tanks were managed at the initial water exchange rate. An additional artificial substrate (5 mm mesh nylon net) area of 16 m^2 (representing two-thirds (66.67 %) of the area of the tank) was used. Therefore, the total surface areas of 0, and 16 m^2 ($S^{0\%}$ and $S^{66.67\%}$, respectively) were tested. Fifteen days before fish stocking in the tanks, the nylon net pieces were put into each assigned tank intended to develop periphyton. On the same day of net placement, a 1 m^3 inoculation of phytoplankton rich water was carried out in all tanks.

Experimental design

A basal commercial diet was formulated to contain (30 % CP ,10.14EE, 5.87ash,3.65CF and 4455k cal/ kg diet Gross energy).The treatments consisted of a 2×3 factorial experiment arrangement to two artificial substrates levels (0 and 66.67%)of tank surface area; ($S^{0\%}$ and $S^{66.67\%}$) and three supplemental probiotics levels(0% without supplementation of probiotic (pro0) and two different commercial probiotic each one supplemented to the basal diet with the recommended level mentioned by the producer 0. 1g/kg for the first (pro1), 2g/kg for the second (pro2) .The first commercial probiotic (Ecobiol Aqua plus®) containing (*Bacillus amyloliquefaciens*) ; while the second (Biogen®)containing (dried natural product composed of Allicin, high unit hydrolytic enzymes, *Bacillus subtilis* and Ginseng extract.) supplemented at recommended levels after doing Viability test to be sure that the products well effective .

All stocked fish were fed with the same artificial diet used during the acclimatization period to apparent satiation, twice a day, for 105 days.

Viability test

The viability test of the two commercial Probiotic were carried out before using according to the method outline by Martin *et al* (1981). The viable contents was determined by containing CFU (Colony Forming Unit), which is considered an indication for the viability of the microorganisms present viable in this commercial Probiotic and so represents its growth promoting effect determination showed the presences of ($6x10^9$) CFU for the first one and ($6x10^7$) for second one .

Chemical analysis of diets and fish:

Diets and whole fish body at the beginning and at the end of the experiment were analyzed for proximate composition. Moisture content, protein, fat and ash according to the standard methods of AOAC (2006).

***Fish performance indices*:**

The growth performance and feed utilization efficiency were calculated as following:

Weight gain (WG) = final weight – initial weight (g/fish).

Specific growth rate (SGR) = 100 (ln W2 – ln W1) / T

Where W1 and W2 are the initial and final weight, respectively, ln represent Natural logarithm and T is the number of days in the feeding period.

Feed conversion ratio (FCR) = dry feed intake (g) / fish live weight gain (g).

Protein efficiency ratio (PER) = 100 (weight gain (g) / protein intake (g)

Protein productive value (PPV) = 100 (protein gain (g) /protein fed (g)).

Energy Retention (ER) = Retained energy in carcass (Kcal)/energy intake (Kcal) ×100.

Statistical analysis

Mean values were reported with a pooled standard error of means (SEM). After confirming normality and homogeneity of variance, the data were analyzed by two-way ANOVA, using periphyton substrate levels and probiotic supplementation as the two factors (SPSS, version 16.0)(2007). Using Duncan's (1955) multiple comparisons to compare between means. Differences were considered significant at ($P<0.05$).

RESULTS AND DISCUSSION

Average values of initial weight (IBW), final body weight (FBW), Body weight gain (BWG); Specific growth rate (SGR) and survival rate (SR%) of Nile tilapia fingerlings fed diets supplemented with probiotics (Pro0 ,Pro1 and Pro2) with or without artificial substrate ($S^{0\%}$ and $S^{66.67\%)}$ are illustrated in Table (1). The initial weight was similar in all treatments group with no significant differences ($p>0.05$). Concerning the artificial substrate treatment, data collected showed that there were significant difference ($p<0.05$) between groups of fish reared in concrete tanks without artificial substrate ($S^{0\%}$) and that reared with artificial substrate ($S^{66.67\%}$) in growth performance parameters ,indicating superiority of the group ($S^{66.67\%}$) which recorded higher values for FW (126.89g), WG (125.02g), SGR (3.85%) and SR 94%. While the other group which reared without substrate ($S^{0\%}$) recorded lower values for the same parameters (107.00, 105.16, 105.16, 3.74 and 91%), respectively.

According to probiotic effect despite substrate effect there were significant differences ($p<0.05$) among the three groups (pro0, pro1 and pro2) in growth performance parameters. Groups received diets supplemented with (pro2) recorded the highest values in all growth parameters (124.33g, 122.48g, 3.85%, 93%) respectively, followed by those received diet supplemented with (pro1) (115.33g, 113.50g, 3.79%, 92%) respectively. The lowest values recorded with the group of fish fed diet without supplementation of probiotic (pro0) (111.17g, 109.29g, 3.74% and 91%), respectively.

The interaction between (substrate x probiotic) showed significant differences ($p<0.05$) among all treatments. Group ($S^{66\%}$pro2)showed the highest values for the pervious growth parameters (133.00g, 131.13g, 3.89% and 94%), respectively .followed by T5, T4, T3, T2 and the lowest values were noticed in the group which fed ($S^{0\%}$ pro0) (98.67g, 96.87g, 3.69% and 90%), respectively .

Feed intake (FI), Feed conversion ratio (FCR), Protein efficiency ratio (PER),protein productive value(PPV) and Energy retention (ER) are tabulated in Table (2) .There were significant differences ($p<0.05$) due to presence of substrate in periphyton based system (231.69g, 1.89, 1.69, 24.67 and 1.00), respectively. Compared with group reared without substrate in the same parameters (201.64, 1.98, 1.63, 22.82 and 9.99), respectively regardless probiotic supplementation.

On the other hand, comparing groups supplemented with probiotics, results demonstrated fluctuated results in feed utilization parameters declared in Table (2).

Table (1): Effect of substrate levels and supplemental probiotics on growth performance of Nile tilapia (*Oreochromis niloticus*).

Treatment	IBW[2] (g)	FBW[3] (g)	BWG[4] (g)	SGR[5] (%)	SR[6] (%)
Substrate level					
$S^{0\%}$	1.84	107.00^{b}	105.16^{b}	3.74^{b}	91^{b}
$S^{66.67\%}$	1.87	126.89^{a}	125.02^{a}	3.85^{a}	94^{a}
SEM[1]	0.03	2.88	2.88	0.02	0.46
P value	0.64	0.00	0.00	0.00	0.00
Probiotic					
Pro0	1.88	111.17^{b}	109.29^{b}	3.74^{b}	91
Pro1	1.83	115.33^{b}	113.50^{b}	3.79^{ab}	92
Pro2	1.85	124.33^{a}	122.48^{a}	3.85^{a}	92
SEM	0.03	2.88	2.88	0.02	0.46
P value	0.72	0.00	0.00	0.04	0.12
Overall mean					
$S^{0\%}$ pro0	1.80	98.67^{e}	96.87^{e}	3.69^{c}	90^{c}
$S^{0\%}$ Pro1	1.90	106.67^{d}	104.77^{d}	3.72b^{c}	90^{c}
$S^{0\%}$ Pro2	1.83	115.67^{c}	113.83^{c}	3.81^{b}	91^{bc}
$S^{66.67\%}$ pro0	1.97	123.67^{b}	121.70^{b}	3.80^{ab}	92^{b}
$S^{66.67\%}$ Pro1	1.77	124.00^{b}	122.23^{b}	3.87^{a}	94^{a}
$S^{66.67\%}$ Pro2	1.87	133.00^{a}	131.13^{a}	3.89^{a}	94^{a}
SEM	0.03	2.88	2.88	0.02	0.46
P value	0.09	0.00	0.00	0.00	0.01

Means in the same column with different superscripts are significantly different ($p<0.05$) ; [1]SEM , Pooled standard error of means ;[2]IBW,Initial body weight;[3]FBWFinal body weight ; [4]BWG, Body weight gain; [5]SGR, specific growth rate;[6] SR, .survival rate

Where, ($S^{0\%}$ and $S^{66.67\%}$) substrate level; (pro0, pro1 and pro2) supplemented level of probiotic

($S^{0\%}$ pro0 ; $S^{0\%}$ Pro1; $S^{0\%}$ Pro2; $S^{66.67\%}$ pro0; $S^{66.67\%}$ Pro1 and $S^{66.67\%}$ Pro2) interaction between treatments (substrate x probiotic).

Table (2): Effect of substrate levels and supplemental probiotics on feed utilization of Nile tilapia (*Oreochromis niloticus*)

Treatment	FI[2] (g)	FCR[3] (g)	PER[4] (g)	PPV[5] (%)	ER[6] (%)
Substrate level					
$S^{0\%}$	201.64^{b}	1.98^{a}	1.63^{b}	22.82^{b}	9.99
$S^{66.67\%}$	231.69^{a}	1.89^{b}	1.69^{a}	24.67^{a}	10.00
SEM[1]	4.90	0.02	0.01	0.51	0.40
P value	0.00	0.00	0.03	0.00	0.99
Probiotic					
Pro0	208.04^{b}	1.96^{a}	1.65	22.95^{b}	8.15^{b}
Pro1	208.39^{b}	1.89^{b}	1.70	25.39^{a}	11.11^{a}
Pro2	233.57^{a}	1.95^{a}	1.64	22.90^{b}	10.72^{a}
SEM	4.90	0.02	0.01	0.51	0.40
P value	0.00	0.07	0.11	0.00	0.00
Overall mean					
$S^{0\%}$ pro0	189.09^{c}	2.02^{a}	1.61^{b}	20.32^{c}	7.23^{c}
$S^{0\%}$ Pro1	196.00^{c}	1.93^{abc}	1.67^{ab}	25.17^{a}	11.65^{a}
$S^{0\%}$ Pro2	219.82^{b}	1.98^{b}	1.62^{b}	22.97^{b}	11.10^{a}
$S^{66.67\%}$ pro0	226.99^{b}	1.90^{bc}	1.68^{ab}	25.57^{a}	9.07^{b}
$S^{66.67\%}$ Pro1	220.78^{b}	1.83^{c}	1.73^{a}	25.62^{a}	10.57^{ab}
$S^{66.67\%}$ Pro2	247.31^{a}	1.93^{abc}	1.66^{ab}	22.83^{b}	10.35^{ab}
SEM	4.90	0.02	0.01	0.51	0.40
P value	0.00	0.02	0.03	0.00	0.00

Means in the same column with different superscripts are significantly different ($p<0.05$) ; [1]SEM , Pooled standard error of means ;[2] FI feed intake;[3]FCR feed conversion ratio ; [4]PER protein efficiency ratio; [5]PPV protein productive value and [6] ER energy retention .

Where,($S^{0\%}$ and $S^{66.67\%}$) substrate level; (No pro,pro1 and pro2) supplemented level of probiotic

($S^{0\%}$ pro0 ; $S^{0\%}$ Pro1; $S^{0\%}$ Pro2; $S^{66.67\%}$ pro0; $S^{66.67\%}$ Pro1 and $S^{66.67\%}$ Pro2) interaction between treatments (substrate x probiotic).

The interaction between (Substrate level and probiotic supplementation) showed significant differences ($p<0.05$) among all the studied parameters. There were significant increase in feed intake in group ($S^{66.67\%}$ pro2) (247.31g) and the lowest feed intake recorded in group ($S^{0\%}$pro0) , ($S^{0\%}$pro1) (189.09, 196.00) with no significant differences between them, the best FCR noticed in group ($S^{66.67\%}$, pro1) while the worst in($S^{0\%}$ pro0) (2.02). Additionally the highest values for PER, PPV and PPV showed in (($p^{66.67\%}$pro1) (1.73,25.62,10.57) respectively as declared in Table (2) .

Results of body composition are illustrated in Table (3). Concerning the substrate treatments no significant difference ($p>0.05$) were noticed for DM, cp and EE content.

There were significant differences ($p<0.05$) in ash content between $S^{0\%}$ (12.76) and $S^{66.67\%}$ (11.75). Data showed also that there were significant differences among groups due to probiotic supplementation.

The highest DM content recorded for the fish fed on pro1 (31.26), with a significant differences between the other group (pro 0, pro1) (28.12, 29.51). The protein content showed no significant differences among all groups. The highest EE content was recorded in pro2 (30.59) followed by pro1 (28.82), the lowest EE recorded at p0 group (24.38). Ash content showed no significant differences between (pro0, pro2)(12.92,12.45) but there were significant differences between the aforementioned groups and pro1(11.38) regardless substrate levels. The results of interaction between substrate levels and probiotic supplementation demonstrate no specific trend was noticed in all body composition content as shown in Table (3).

Table (3): Effect of substrate levels and supplemental probiotics on body chemical compositions of Nile tilapia (*Oreochromis niloticus*).

Treatment	DM[2] (%)	CP[3] (%)	EE[4] (%)	ASH[5] (%)
Substrate level				
$S^{0\%}$	29.51	47.03	28.34	12.76^{a}
$S^{66.67\%}$	29.76	48.61	27.52	11.75^{b}
SEM[1]	0.50	0.48	0.73	0.26
P value	0.69	0.09	0.23	0.00
Probiotic				
Pro0	28.12^{b}	49.13	24.38^{c}	12.92^{a}
Pro1	31.26^{a}	47.29	28.82^{b}	11.38^{b}
Pro2	29.51^{b}	47.04	30.59^{a}	12.45^{a}
SEM	0.50	0.48	0.73	0.26
P value	0.00	0.14	0.00	0.00
Overall mean				
$S^{0\%}$ pro0	26.36^{c}	47.77^{ab}	23.71^{b}	14.06^{a}
$S^{0\%}$ Pro1	31.79^{a}	46.90^{b}	30.24^{a}	11.71^{bc}
$S^{0\%}$ Pro2	30.37^{ab}	46.43^{b}	31.07^{a}	12.52^{c}
$S^{66.67\%}$ pro0	29.89^{b}	50.50^{a}	25.05^{ab}	11.79^{bc}
$S^{66.67\%}$ Pro1	30.73^{ab}	47.68^{ab}	27.41^{b}	11.06^{c}
$S^{66.67\%}$ Pro2	28.66^{bc}	47.66^{ab}	30.11^{a}	12.39^{b}
SEM	0.50	0.48	0.73	0.26
P value	0.01	0.19	0.00	0.00
Treatment				

Means in the same column with different superscripts are significantly different ($p<0.05$) ; [1]SEM , Pooled standard error of means ;[2] DM dry matter; [3] CP crude protein ;[4] EE ether extract and [5] ASH ash content

Where,($S^{0\%}$ and $S^{66.67\%}$) substrate level; (pro0,pro1 and pro2) supplemented level of probiotic

($S^{0\%}$ pro0 ; $S^{0\%}$ Pro1; $S^{0\%}$ Pro2; $S^{66.67\%}$ pro0; $S^{66.67\%}$ Pro1 and $S^{66.67\%}$ Pro2) interaction between treatments (substrate x probiotic).

The results of this study indicated that the addition of artificial substrate ($S^{66.67\%}$) increased tilapia growth performance parameters when combined with pro2 (Biogen®) supplementation. The final body weight (FBW) increased by (18.8%) than the fish group with no supplementation of probiotic and artificial substrate. This value is higher than the value reported for tilapia being 16% (Abo-Taleb 2014) and less than the value found for carp monoculture being,30 to 115% (Wahab *et al*., 1999a; Keshavanath and Gangadhar,2005) and 30% to 210% in carp polyculture (Azim and wahab,2005) under semi

intensive periphyton based system. Since periphyton in periphyton based system provided significant additional food for Nile tilapia juveniles (Saker *et al*., 2015), and tilapia is regarded as an omnivorous species and capable of feeding on benthic and attached algal and detritus aggregates (Dempster *et al*., 1993; Azim *et al*., 2003b). Similarly, (Uddin *et al*., 2007), found that tilapias can graze on the substrates in the experimental ponds. Therefore, the addition of substrate offers considerable improvement in performance of fish under semi intensive culture system. Better growth performance may be attributed due to 1) the additional shelter provided the substrate allows more of the resources to flow into fish biomass, (2) the new primary production and attached benthic secondary production by the artificial substrate support a new food web, which is reflected on increasing biomass(Miller and flace 2000) . These in agreement with the finding of (Audelo-Naranjo *et al*., 2011; Anand *et al*., 2013) in shrimp culture,(Anand *et al*., 2014) on tiger shrimp.

Better growth recorded in tilapia fed diets supplemented with probiotic in periphyton system might be attributed to presence of microorganisms and the viability of these microorganisms. This is in agreement of the results of (Nonwachai *et al*., 2010) their result declared improvement in growth performance for shrimp fed micro algae supplemented diet. They explained their results attributed to unknown growth promoters or higher digestive enzymes. Mridula *et al* (2005) suggests that periphyton ingestion increases enzyme activities of intestinal and hepatopancreatic protease, lipase and intestinal amylase in tilapia fingerlings. Another factor may induce good performance in fish in periphyton based system is the size of the submerged area available for periphyton growth (Asaduzzaman *et al.,* 2010). Since the higher area, the greater the benefits from periphyton will be (Uddin *et al*., 2009). In addition to the role of periphyton played in improving fish performance, it may play another role in alleviating the environmental impacts on the aquatic environment (Saker *et al*., 2015) this results is in agreement with that found by Troell and Berg (1997) suggested that the average flix of particulate nutrient (particularly ammonium and phosphates) under intensive tilapia cages were up to 22 times greater than in cage free areas. , improve water quality and mitigates negative effects of overcrowding.

The present results were in agreement with the results obtained by many authors. Renuka *et al*. (2013) suggested that the incorporation of probiotic in common carp diets stimulated fish growth and digestion as micro biota colonization enzymes that hydrolyze complex molecules, facilitate better digestion and absorption of macronucleus resulting in higher protein and energy deposition in the body tissues. In these aspects, Agouz and Anwer (2011) pointed out to the improvement of digestion and metabolism in the fish body due to the presence of the *Bacillus* in the probiotic Biogen®, moreover the prevention of pathogenic bacteria colonies in fish gut. Faramarzi *et al*. (2011) was in accordance with the results obtained in the present work, where they found that the addition of 0.1% probiotics (*Bacillus subtilis* c-3102 spores) in common carp fry diets improved fish growth and mitigated the effects of stress factors. In this particular, diets supplemented mix of Lactobacillus SP. spores resulted in improving growth performance of Striped mullet (*Mugil cephalus*) significantly than those fed the control basal diets (El-Tawil *et al*., 2012)

The positive effect of probiotic was also observed in several kinds of fish, in Sea bream (*Sparus aurata* L.) (Avella *et al*., 2010) and Large croaker (*Larimichthys crocea*) (Ai Q 2011) Common snook and Red drum (Hauville *et al*., 2016). The high viability of microorganisms in the probiotic product may be considered as another reason for the positive and better effect of the second probiotic (pro2). This fact was proved by the studies carried out by Mohapatra *et al* (2012) who found that incorporation of live probiotic microorganisms (*Lactobacilis lactis* and *Bacillus subtilus*) resulted in maximum growth performance in rohu (*Labeo rohita*) fingerlings in comparison with some combinations of inactivated probiotics. Other similar results were also observed for Nile tilapia (Lara-Flores *et al*., 2003), L. rohita (Ghosh *et al*., 2003) *Cyprinus carpio* (Ramakrishnan *et al*., 2008).

In semi-intensive systems, the availability of periphyton for fish can reduce the importance of supplemental feed allowance and improve feed conversion ratio, due to fish partially satisfied by ingestion of periphyton (Abo-Taleb 2014 and Reboucas *et al*., 2012). However, completely withdrawing supplemental feed may have negative effects on fish growth (Milstein *et al*., 2009). Thus, the quality and quantity of supplemental feed are critical to profitability.

Consequently, our results were in the same trend found in the previous studies about the efficiency of feed utilization, where several workers recorded different degrees of improvement in feed and protein utilization parameters in diets supplemented with probiotic or growth promoters, which reflected the increasing growth rate (Renuka *et al*., 2013and Lemieux *et al*., 1999). Faramarzi *et al*., (2011) found also improvement in the feed utilization in Common carp fed diet supplemented with 0.1% probiotics (*Bacillus subtilis* c-3102 spores). Similar positive effects in feed utilization were recorded in many fish species as mentioned previously during the discussion of growth performance.

The improvement in body composition of Nile tilapia fed probiotic is a significant evidence of the improvement in general health condition of the reared fish. This positive effect in body composition of Nile tilapia may be due to improving of growth performance, enhance the metabolism and energy of fish body cells and raise the efficiency of feeds (Mehrim 2010).The results of body composition in this study were in close agreement with Mohamed *et al*. (2007) for tilapia. On the other hand, Eid and Mohamed (2008) found no statistical differences were observed in whole body moisture, crude protein, ether extract and ash for mono sex *O. niloticus* fingerlings fed diets containing different levels of commercial feed additives.

This study indicates that Nile tilapia fed diets with supplemental probiotic in periphyton based system may improve growth performance ,feed utilization and body composition in Nile tilapia than using these supplementation without periphyton based system , in concomitant with our results (Garcia *et al*., 2013, 2016) suggested that periphyton should be making part of the diet of fish and the cage nets can act as a substrate for periphyton growth at commercial scale , under real environmental conditions and management.

CONCLUSION

It could be concluded that supplementation of probiotic (Biogen®) and artificial substrate led to improvement of periphyton growth, achieved an improvement in sustainability performance of tilapia production under limited water exchange. Additional research is required to determine the specific microbial sources under different feeding strategy.

REFERENCES

Abo-Taleb, M.A.A. (2014). Application of different feeding systems for production of Tilapia in green and clear water. Msc., Animal Production Department, Faculty of Agriculture, Cairo University.

Abo-Taleb, M.A.A.; M.A. El-Sherbiny; M. A. Elnady; H. A. Hassanien; A. Suloma and A.M. Tahoun (2014). Combined effects of periphyton substrate and alternate day feeding strategy on growth performance of Nile tilapia *Oreochromis niloticus* L. under green water tank culture. Egyptian Journal of Aquaculture. 4, (2): 13-26.

Agouz, H.M. and W. Anwer (2011). Effect of Biogen® and growth performance of Myco-Ad on the growth performance of common carp (*Cyprinus carpio*) fed a mycotoxin contaminated Aqua feed .Journal of Fisheries and Aquatic Science, 6(3): 334-345.

Ai, Q.; H. Xu; K. Mai; W. Xu; J. Wang and W. Zhang (2011). Effects of the dietary supplementation of *Bacillus subtilis* and fructooligoscharide on growth performance, survival, non specific immune response and disease resistance of juvenile large yellow croaker, *Larimichthys crocea*. Aquaculture, 317:155-161.

Amisah, S.; D. Adjei-Boateng and D. D. Afianu (2008). Effects of bamboo substrate and supplementary feed on growth and production of the African catfish, *Clarias gariepinus*. Journal of Applied and Environmental Management, 12 (2): 25-28.

Anand, P. S.; M. P. S. Kohli; S. D. Roy; J. K. Sundaray; S. Kumar; A. Sinha; G.H. Pailan and M. K. Sukham (2014). Effect of dietary supplementation of periphyton on growth, immune response and metabolic enzyme activities in *Penaeus monodon*. Aquaculture Research, 46 (9): 2277-2288

Anand, P.S.S.; S. Kumar; A. Panigrahi; T.K. Ghoshal; J.S. Dayal; G. Biswas; J.K. Sundaray; D. De; R.A. Raja; A. D.; S. M. Pillai and P. Ravichandran (2013). Effects of C: N ratio and substrate integration on periphyton biomass, microbial dynamics and growth of *Penaeus monodon* juveniles. Aquaculture International, 21: 511–524.

AOAC. (2006) Official Methods of Analysis, 18th ed. Association of Official Analytical Chemists, Arlington, VA, USA

Arnold, S. J.; F. E. Coman; C. J. Jackson and S. A. Groves (2009). High-intensity, zero water-exchange production of juvenile tiger shrimp, Penaeus monodon: an evaluation of artificial substrates and stocking density. Aquaculture, 293, 42-48.

Asaduzzaman, M.; M.M. Rahman; M.E. Azim; M.A. Islam; M.A. Wahab; M.C.J. Verdegem and J. A. J. Verreth (2010). Effects of C/N ratio and substrate addition on natural food communities in freshwater prawn monoculture ponds. Aquaculture, 306:127–136.

Audelo-Naranjo, J.M.; L.R. Martinez-Cordova; D. Voltolina and S. Gomez-Jimenez (2011). Water quality, production parameters and nutritional condition of *Litopenaeus vannamei* (Boone, 1931) grown intensively in zero water exchange mesocosms with artificial substrates. Aquaculture Research, 42:1371–1377.

Avella, M.A.; G. Gioacchini; O. Decamp; P. Makridis; C. Bracciatelli and O. Carnevali (2010). Application of multi-species of Bacillus in sea bream larviculture. Aquaculture, 305: 12–1

Azim, M. E.; T. Asaeda; M.C.J. Verdegem; A.A. van Dam and M.C.M. Beveridge (2005). Periphyton structure, diversity and colonization. In: Periphyton: Ecology, Exploitation and Management (ed. by M.E. Azim, M.C.J.Verdegem, A.A. van Dam & M.C.M. Beveridge), pp. 207–222. CABI Publishing, UK.

Azim, M. E.; M.A. Wahab; A.A. van Dam; M.C.M. Beveridge; A. Milstein and M.C.J. Verdegem (2001). Optimization of fertilization rate for maximizing periphyton production on artificial substrates and the implications for periphyton-based aquaculture. Aquaculture Research, 32: 749-760.

Azim, M.E.; M.C.J. Verdegem; I. Mantingh; A.A. Van Dam and M.C.M. Beveridge (2003b). Ingestion and utilization of periphyton grown on artificial substrates by Nile Tilapia, *Oreochromis niloticus* L. Aquaculture Research, 34: 85-92.

Azim, M.E.; M.C.J.Verdegem; M. Singh; A.A. Van Dam and M.C.M. Beveridge (2003a). The effects of periphyton substrate and fish stocking density on water quality, phytoplankton, periphyton and fish growth. Aquaculture Research, 34: 685-695**.**

Balcazar, J.L.; I. de Blas; I. Ruiz-Zarzuela; D. Cunningham; D. Vendrell and J.L. Muzquiz (2006). The role of probiotics in Aquaculture, Vet. Microbiol., 114: 173–186.

Cruz, P. M.; Ibanez, A. L. Hermosillo, M. and Saad, H. C. R. (2012): Use of Probiotics in Aquaculture. International Scholarly Research Network (ISRN) Microbiology Volume PP. 13. Open access journal.

Dempster, P.W.; M.C.M. Beveridge and D.J. Baird (1993). Herbivory in the tilapia *Oreochromis niloticus*: A comparison of feeding rates of phytoplankton and periphyton. Journal of Fish Biology, 43: 385-392.

Duncan, D. (1955). Multiple range tests and multiple F tests. Biometrics, 11: 1- 42.

Eid, A. H. and K. A. Mohamed (2008). Effect of using probiotic as growth promoters in commercial diets for mono sex Nile tilapia (*Oreochromis niloticus*) fingerlings. 8th international symposium of tilapia in aquaculture, Egypt. 241-253.

El-Tawil NE, Amer TN, Ahmed MH, Seden ME (2012). Effect of different levels of probiotic (Lactobacillus sp.,) on fish performance, survival and feed efficiency of striped mullet Mugil cephalus fed on all plant diet. Egyptian Journal of Nutrition and Feeds, 15(1):193-202.

Faramarzi, M.; S. Kiaalvandi and F. Iranshahi (2011). The effect of probiotics on growth performance and body composition of common carp (*Cyprinus carpio*). Journal of Animal and Veterinary Advances, v. 10, n. 18, p.24

Garcia, F.; D. M. Romera; N.S. Sousa; I. Paiva-Ramos and E.M. Onaka (2016). The potential of periphyton-based cage culture of Nile tilapia in a Brazilian reservoir. Aquaculture, 464:229–235

Garcia, F.; D.M. Romera; K.S. Gozi; E.M. Onaka; F.S. Fonseca; S.H.C. Schalch; P.G. Candeira; L.O. Guerra; F.J. Carmo; D.J. Carneiro; M.I.E. Martins and M.C. Portella (2013). Stocking density of Nile tilapia in cages placed in a hydroelectric reservoir. Aquaculture, 410–411:51-56.

Ghosh, K.; S. K. Sen and A. K. Ray (2003). Supplementation of an isolated fish gut bacterium, *Bacillus circulans*, in formulated diets for rohu, *Labeo rohita*, fingerlings. Israli Journal of Aquaculture, Bamidgeh, 55:13-21.

Gonzalez, R. J. (2012). The physiology of hyper-salinity tolerance in tellers fish: a review. Journal of Compartive Physiology B., 182: 321–329.

Hasan, M. N.; M. S. Rahman; M. F. Hosen and M. A. Bashar (2012). Effects of addition of tilapia on the abundance of periphyton in freshwater prawn culture ponds with periphyton substrates Journal of Bangladesh Agriculture University, 10 (2): 313–324.

Hauville, M. R.; J. L. Zambonino-Infante; J. Gorden Bell; H. Migaud and K.L. Main, (2016). Effects of a mix of Bacillus sp. As a potential probiotic for Florida Pompano, Common Snook and red drum larvae performances and digestive enzyme activities. Aquaculture Nutrition, 22: 51-60.

Hem, S. and J.L.B. Avit (1994). First results on 'acadja-enclos' (bamboo reefs) as an extensive aquaculture system, Cote d'Ivoire (West Africa). Bulletin of Marine Science 55, 1040–1051.

Huchette, S. M. H. and M. C. M. Beveridge (2003). Technical and economical evaluation of periphyton-based cage culture of tilapia (*Oreochromis niloticus*) in tropical freshwater cages. Aquaculture, 218: 219–234.

Huchette, S.M.H.; M.C.M. Beveridge; D.J. Baird and M. Ireland (2000). The impacts of grazing by tilapias (*Oreochromis niloticus* L.) on periphyton communities growing on artificial substrate in cages. Aquaculture, 186: 45–60.

Hussein M.S.; A. Zaghlol; N. F. Abd ElHakim; M. El Nawsany and Hanan A. Abo- State (2016). Evaluation the effect of different growth promoters on the growth performance, feed utilization and body composition of Common carp (*Cyprinus carpio*) reared in earthen ponds. Journal of fisheries and aquatic science, 11: 370-377.

Keshavanath, P. and B. Gangadhar (2005). Research on periphyton-based aquaculture in India. In *Periphyton: Ecology, exploitation and management*, ed. M. E. Azim, M. C. J. Verdegem, A. A. Van Dam, and M. C. M. Beveridge, 223–236. Wallingford, UK: CABI Publishing.

Keshavanath, P.; B. Gangadhar; T.J. Ramesh; A. A. Van Dam; M. C. M. Beveridge and M. C. J. Verdegem (2004). Effects of bamboo substrate and supplemental feeding on growth and production of hybrid red tilapia fingerlings (*Oreochromis mossambicus* x *Oreochromis niloticus*). Aquaculture, 235:303–314.

Keshavanath, P.; B. Gangadhar; T.J. Ramesh; J. M. Van Rooij; M.C.M. Beveridge; D.J. Baird; M.C.J. Verdegem and A.A. Van Dam (2001). Use of artificial substrates to enhance production of freshwater herbivorous fish in pond culture. Aquaculture Research, 32: 189–197.

Lara- Flores, M.; M.A. Olvera–Novoa; B.E. Guzmán–Méndez and W. López-Madrid (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.

Lemieux, H.; P. Blier and J. D. Dutil (1999). Do digestive enzymes set a physiological limit on growth rate and food conversion efficiency in the Atlantic cod (*Gadus morhua*). Fish Physiology and Biochemistry, 20:293-303.

Martin, p. A. W.; Lohr, J. R. and Dean, D. H. (1981): Transformation of Bacillus thuringiensis protoplasts by plasmid deoxyribonucleic acid. Journal of Bacteriology, 145: 980 – 983.

Mehrim, A.I. (2010). Effect of dietary supplementation of Biogen® (commercial probiotic) on monosex Nile tilapia *Oreochromis niloticus* under different stocking densities. Journal of Fisheries and Aquatic Science,4:261-273.

Miller, M.W. and A. Falace (2000). Evaluation methods for trophic resource factors-nutrients, primary production, and associated assemblages. In: Seaman Jr., W. (Ed.), Artificial Reef Evaluation with Application to Natural Marine Habitats. CRC Press, pp. 95–126.

Milstein, A.; D. Joseph; Y. Peretz and S. Harpaz (2005). Evaluation of organic tilapia culture in periphyton-based ponds. Israeli Journal of Aquaculture–Bamidgeh, 57:143–155.

Milstein, A.; Y. Peretz and S. Harpaz (2009). Culture of organic tilapia to market size in periphyton based ponds with reduced feed inputs. Aquaculture Research, 40:55–59.

Mohamed, K.A.; B. Abdel Fttah and A.M.S. Eid (2007). Evaluation of using some feed additives on growth performance and feed utilization of monosex Nile tilapia (*Oreochromis niloticus*) fingerlings. Agriculture Research Journal of Suez Canal University, 7: 49-54.

Mohapatra, S.; T. Chakraborty; A.K. Prusty; P. Das; K. Paniprasad and K.N. Mohanta (2012): Use of different microbial probiotics in the diet of rohu, Labeorohita fingerlings: Effects on growth, nutrient digestibility and retention, digestive enzyme activities and intestinal microflora. Aquaculture Nutrition. 18:1-11.

Mridula, R. M.; J. K. Manissery; P. Keshavanath; K. M. Shankar; M. C. Nandeesha and K. M. Rajesh (2005). Effects of paddy straw and sugarcane bagasse on water quality, bacterial biofilm production and growth and survival of rohu, *Labeo rohita* (Hamilton). Aquaculture Research, 36: 635–642.

Nonwachai, T.; W. Purivirojkul; C. Limsuwan; N. Chuchird; M. Velasco and A. K. Dhar (2010). Growth, non-specific immune characteristics, and survival upon challenge with Vibrio harveyi in Pacific white shrimp (*Litopenaeus vannamei*) raised on diets containing algal meal. Fish and Shellfish Immunology, 29: 298–304.

Ramakrishnan, C. M.; M. A. Haniffa; M. Manohar; J. M. Dhanara; A. Jesu Arockiaraj; S. Seetharaman and S.V. Arunsingh (2008). Effects of probiotics and spirulina on survival and growth of juvenile Common carp *Cyprinus carpio*. Israli Journal of Aquaculture Bamidgeh, 60:128-133.

Rebouças V.T.; N.N. Caldini; D. D. Cavalcante; F. J. R. Da Silva and M. V. Do Carmo (2012). Interaction between feeding rate and area for periphyton in culture of Nile tilapia juveniles. Acta Scientiarum. Animal Sciences Maringá, v. 34, n. 2, p. 161-167.

Renuka, K.P.; M. Venkateshwarlu; A.T. Ramachandra Naik and S. M.Prashantha Kumara (2013). Influence of probiotics on growth performance and digestive enzyme activity of common carp *Cyprinus carpio*. International Journal of Current Research, 5 (7): 1696-1700

Rezoanul, H.; M. A. Islama; M. Abdul Wahabb; M.D. Enamul Hoqc; M. M. Rahmand and M. E. Azimea (2016). Evaluation of production performance and profitability of hybrid redtilapia and genetically improved farmed tilapia (GIFT) strains in thecarbon/nitrogen controlled periphyton-based (C/N- CP) on-farm prawn culture system in Bangladesh. Aquaculture Reports 4:101–111.

Ringø, E., Z. Zhou, J.L.G. Vecino; S. Wadsworth, J. Romero, A. Krogdahl, R.E. Olsen, A. Dimitroglou, A. Foey, S. Davies, M. Owen, H.L. Lauzon, L.L. Martinsen, P. De Scheryver, P. Bossier, S. Sperstad and D.L. Merrifield, 2015. Effect of dietary components on the gut microbiota of aquatic animals. A never-ending story?. Aquaculture Nutrition, 22 (2): 219-282.

Ringø, E.; R.E.O. Joe; L.G. Vecino; S. Wadsworth and S. Song (2012). Use of immunostimulants and nucleotidesim aquaculture, a review. Journal of Marine Science Research Development, 2:1-22.

Sakr E.M.; S. M. Shalaby; E.A. Wassef; A. M. El- Sayed and A. I. Abdel Moneim (2015). Evaluation of periphyton as a food source for Nile tilapia (*oreochromis niloticus*) juveniles fed reduced protein levels in cages. Journal of Applied Aquaculture, 27:1, 50-60.

SPSS. (2007). Statistical Package for Social Science (for Windows). Release 16 copyright, SPSS Inc., Chicago, USA.

Troell, M. and H. Berg (1997). Cage fish farming in the tropical Lake Kariba, Zimbabwe: Impact and biogeochemical changes in sediment. Aquaculture Research, 28:527–544.

Uddin, M. S.; M. E. Azim; M. A. Wahab and M. C. J. Verdegem (2009). Effects of substrate addition and supplemental feeding on plankton composition and production in Tilapia (*Oreochromis niloticus*) and water prawn (*Macrobrachium rosenbergii*) polyculture. Aquaculture, 297(1–4): 99–105.

Uddin, M.S.; S.M.S. Rahman; M.E. Azim; M.A. Wahab; M.C.J. Verdegem; J.A.J. Verreth (2007). Effects of stocking density of Nile tilapia (Oreochromis niloticus) and fresh water prawn (Macrobrachium rosenbergii) -based systems. Aquacult. Res., 38: 1759–1769.

Van Dam, A.A.; M.C.M. Beveridge; M.E. Azim and M.C.J. Verdegem (2002). The potential of fish production based on periphyton. Rev. Fish Biol. Fish., 12: 1-31.

Verschuere, L.; G. Rombaut; P. Sorgeloos; W. Verstraete (2000). Probiotic bacteria as biological control agents in aquaculture. Microbiology and molecular biology reviews 64: 655- 671.

Wahab, M. A.; M. A. Mannan; M. A. Huda; M. E. Azim; A. G. Tollervey and M. C. M. Beveridge (1999a). Effect of periphyton grown on bamboo substrates on growth and production of Indian major carp, rohu (*Labeo rohita*). Bangladesh Journal of Fisheries Research 3:1–10.

Wahab, M. A.; M. E. Azim; M. H. Ali; M. C. M. Beveridge and S. Khan (1999b). The potential of periphyton-based culture of the native major carp calbaush, *Labeo calbasu* (Hamilton). Aquaculture Research 30:409-41.

المساهمات الإيجابية للإضافات من البروبيوتك والركائز الإصطناعية على أداء النمو والاستفادة الغذائية لإصبعيات البلطى النيلى

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أجريت هذه الدراسة لدراسة التأثيرات المشتركة لاستخدام الركائز التى تنمو عليها الطحالب الملتصقة مع استخدام نوعين من المنشطات الحيوية (البروبيوتيك) كاضافات غذائية علفية وتأثير ذلك على استجابة الأداء الانتاجى لعدد (12960) اصبعية من اصبعيات البلطى النيلى بوزن ابتدائى (1.86جم) . وقد أجريت هذه الدراسة لمدة 105 يوم باستخدام أحواض خرسانية (18حوض) بابعاد (8م طول ، 3م عرض ، 1م عمق). وزعت الاصبعيات فى تجربة عاملية (2*3) كالاتى : مستويين مختلفين للركائز التى تنمو عليها الطحالب الملتصقة (غزل هابات النايلون) (صفر ، 66، 67%) و3 مستويات من الاضافات الغذائية (بدون اضافة و النوع الاول من البروبيوتك؛ والنوع الثانى من البروبيوتك) وكل معاملة لها 3 مكررات.

وقد لوحظ وجود اختلافات معنوية بالنسبة لمقاييس النمو والاداء الانتاجى و الاستفادة الغذائية وحققت أفضل النتائج فى التأثير المتداخل أى عند استخدام هذه الركائز بنسبة 66.67% مع النوع الثانى من البروبيوتك

وتخلص التجربة الى امكانية استخدام الركائز التى تنمو عليها الطحالب الملتصقة مع النوع الثانى من البروبيوتك (البيوجين) لمساهمتها الجيدة كجزء من النظام الغذائى لاصبعيات البلطى النيلى مما يحقق الاستدامة فى انتاج اسماك البلطى تحت ظروف تغيير محدود للمياه.