Rearing studies on *Oreochromis niloticus* to evavaluate *bacillus subtilis* potential in growth performance and some physiological parameters

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ABSTRACT

The present study was carried out to clarify the effect of adding *Bacillus subtilis* as a probiotic and growth promoter, to evaluate its potential for *Oreochromis niloticus* fingerlings growth performance. Used fish were divided into two groups according to their initial body weight; small size fish with average initial body weight 3.5 g. and length 3.7 cm. and big size fish with aver. IBW 15.3 g. and length 13.5 cm. Each fish group, was divided to four subgroups; T_1 was the control that offered the basal diet only, while the other three subgroups ; T_2 , T_3 and T_4 were fed on basal diets that contained *B. subtilis* solution with three different levels 5,10 and 15.0 ml /l00 g diet, contained 10^8 CFU ml^{-1} respetively. Experimental period was 90 days in a three replicates.

The results show that growth performance in small size *O. niloticus* (FBW), (WG), ADG and SGR values increased significantly with increasing *B. subtilis* levels. Differences, were observed with significantly higher values, recorded from the highest *B. subtilis* level, comparina to the control. Feed conversion rate showed decreases with the increasing *B. subtilis* level.

Survival had best values for fish groups offered diets with adding *B. subtilis*. Serum analysis, total protein, albumin and globulin had the same pattern. Maximum values were recorded from T_4 in contrast to the control (T_1). Big size fish showed same results of growth performance, with significant differences between fish groups offered diets with *B. subtilis* and the control diet groups. Differences were significantly higher from treatment (4) compared to the control. F.C.R showed the lowest value from T_4, while T_1 recorded the highest value.

Big size fish showed the same results in FBW, WG, ADG and S.G.R. Differences were observed with significantly higher values from fish groups offered diet contained high *B. subtilis* level. Compared to the control, F.C.R. showed the best and lowest value from treatment (4), in contrast to the control which recorded the highest value.

Serum analysis (T.P.C), (A) albumen and (G) globulin increased significantly with the increase in *B. subtilis* level. Treatment (4) had the best values in contrast to the control. In both small and big size fish. A: G rate decreased with the increasing *B. subtilis* level, best value was recorded from treat. (4), while T_1 recorded the highest value. Small size fish had the best results and were more benefited from adding *B. subtilis* to their diets than those in big

size fish. These results indicted that *B. subtilis* can be used effectively as a commercial product for enlrancing growth performance in aquaculture.

Key words: Probiotic, *Bacillus subtilis,* growth performance, *Oreochromis niloticus,* aquaculture.

INTRODUCTION

In the last several decades, a number of trials have been conducted in different live stock with various indigenous and exogenous useful microorganisms called probiotics to boost gastrointestinal microflora to light against infectious diseases (Verschuere *et al.,* 2000). Probiotics are beneficial inter organisms that protect the host from diseases (Fuller, 1992). Several, scientists showed that the addition of probiotic in fish diets, improve growth performance. The results of Jena *et al.* (1996) revealed that Catla & Rohu fry fed diets with probiotics had significantly increased body weight and length compared with fry fed diets without adding probiotics. Thompson *et al.* (1999) studied the importance of microorganisms as live probiotic in the feed of *Penaeus paulensis* larvae. They reported that larvae fed only bacterial preparation survived 3 days longer than those cultured in filtered sea water.

Abd EI-Hakim *et at.* (2006) revealed that adding some sources of probiotic, such as Biogen®, Biovit and Bioaction to tilapia's fry feeding increased growth performance parameters, than those fed on the control diets. F AO/WHO (2001) stated that probiotics are live microorganisms, which when consumed in adequate amounts, affer a health benefit for the host. Balcàzar *et at.* (2006) studied the role of probiotics in aquaculture and preface it as a relation between specific immune system stimulating and doses of probiotic, it has been demonstrated that oral administration of *Clostridium butyricum* bacteria to rainbow trout enhanced the resistance of fish to vibriosis, by increasing the phagocytic activity of leucocytes (Sakai *et al.,*1995). Rengpipat *et al.* (2000) mentioned that the use of *Bacillus* sp. (strain 511) provided disease protection by activating both cellular and humeral immune defenses in tiger shrimp.

There are some consideration of regulations on probiotics the basis on which the utilization of feed additives was consolidated has been modified in the European Union. In terms of the additives used in feed products. Watson *et al.* (2008) studied the need, principal and mechanisms of action of the probiotic in aquaculture. It has been widely published that a probiotic must prossess certain properties, it was proposed in order to aid correct establishment of new effective and safe products.

The aim of this present study was to evaluate *Bacillus subtilis* potential in *O. niloticus* growth performance and serum parameters changes.

MATERIAL AND METHODS

1) **Study Site:** The present experiment was carried out in Barrage Fish Research station which belongs to the National Institute of Oceanography & Fisheries in concrete ponds filled with underground water, where each pond area was 8.0 m^3 (4x2x1 m).
2) **Tested fish:** *Orechromis niloticus* fingerlings were divided into two groups: group1, with average initial body weight $3.5^{\pm 0.02}$ g, initial length $3.7^{\pm 0.01}$ cm, group2, with an average initial weight $15.3^{\pm 0.07}$ g, length $13.3^{\pm 0.03}$ cm, stocking density was 7 fish /m^3 or 56 fingerlings / pond.
3) **Used diets:** Fish were fed on commercial supplementary diets containing 30% crude protein and 4870 k. cal/kg diet offered to fish at a rate of 6.0 % of total body weight/day, for six days a week at two times. Table (1) illustrates the diet composition and chemical proximate analysis of the diet ingredients.

Table (1) Composition and proximate analysis of basal diet containing 30% crude protein.

Items / Ingredients	Total weight in diet%				Chemical composition%	
	T1	T2	T3	T4		
Soya bean meal.	20	20	20	20	Crude protein %	30.3
Cotton seed cake.	19.7	19.7	19.7	19.7	ether extract %	8.75
Fish meal.	10.2	10.2	10.2	10.2	Crude fiber %	7.1
Rice Bram.	10	10	10	10	Ash%	10.3
Wheat Bram.	39	39	39	39	Nitrogen free extract	42.5
Oil.	1	1	1	1	Gross-energy k-cal/kg diet	4875.0
Vitamin-mineral	0.1	0.1	0.1	0.1		
Total *Bacillus subtilis* ml/100 g. diet.	0	5	10	15		

4) **Experimental technique:** used fish were divided into two groups according to their initial body weight to small and big size fish; each fish group was divided into four subgroups, T_1 was the control fed on commercial diets without adding *B. subtilis*, the other subgroups T_2 ,T_3 and T_4 were fed diets containing three concentrations 5,10 and 15 ml with 10^8 CFU ml^{-1} /100g. diet *B. subtilis* solution respect.
5) **Mass culture of *B. subtilis*:** A pure culture of` *B. subtilis* was inoculated into a conical flask (500 ml) containing nutrient broth and incubated at 30°c for 24 h. in a shaker incubator. The culture was centrifuged at 10000 rpm for 20 min. at 4°c and the super natant was discarded, while the pellet was resuspended in phosphate-buffered saline (PBS, pH 7.2). The suspension

was similarly washed and recentrifuged 3-4 times and then quantified by the spread plate technique (nutrient agar, incubated at 30°c for 24 h. Purified quantified Bacteria were kept at 4°c in suspended form and were used for feed preparation on request.

6) **Serum chemistry parameters:** Blood was collected from the caudal region of fish without rinsing the syringe with anticoagulants and collected in an Eppendorf tube. The blood was allowed to clot for 45 minutes in an inclined position at room temperature, followed by 30 min. incubation at 4°c and then centrifuged at 300 rpm for 10 min. at 4°c, serum was collected in sterilized Eppendorf tube and analyzed for different serum parameters in an AR 601 semi-automatic analyzer (Qualigens Diagnostic kit). For all serum parameter Qualigens kits were used.

   The parameters that were analyzed by a semi-automatic analyzer (AR 601, Qualigens Diagnostics kits) were:

   1- Total protein biuret methods using buffered dye reagent and biuret reagent.
   2- Albumin (bromocrysol green binding method).
   3- Alkaline phosphatase (Kinetic- colorimeter method).
   4- Aspartate amino- transferase (AS T_1 modified. IFCC method).

7) S tatistical analysis: Significant differences among treatment groups were tested by one-way analysis of variance (ANOY A) and the comparison of any two mean values was made by Duncan's multiple range test, a significance level of ($p<0.05$) was used according to Snedecor and Cochran (1967).

RESULTS

Growth performance parameters

All growth parameters of Nile Tilapia fingerling as affected by adding *Bacillus subtilis* are presented in Table (2). Results of final body weight had significant differences between the control and other treatments T_2 , T_3 and T_4 which recorded average values of 20.2 , 22.5 and 28.5 respectively, with respect to the control value (18.7) for the small sized fish, while big sized groups T_2 , T_3 and T_4 recorded 46.3, 55.5 and 80.5 g./ fish, while T_1 value was 32.3g , total weight gain (T.W.G) followed the same pattern as in FBW, where fish groups of small size T_4 with 15 ml contained 10^8 CFU/ml^{-1} *Bacillus subtilis* was significantly ($p<0.05$) superior in weight gain which recorded 25.3 g. / fish and followed in a significant descending order by T_3, T_2 and T_1 , which recorded 19.0 , 16.7 and 15.2 g. /fish respectively, It is illustrated in Table (2) that weight gain of the big size fish had a significant difference ($p<0.05$), T_4 which fed diet contained the highest level of *B. subtilis* concentration was the superior in weight gain which recorded 65 g / fish followed in a significant descending order by T_3 , T_2 and T_1 with values of 40.2, 31.0 and 17. g. /fish respectively).

Average daily gain is presented in Table (2); the highest average daily gain, ($p<0.05$) was recorded in T_4 in both small and big sized fish. The small size recorded 0.27 g/fish day, followed in a significant ($p<0.05$g) the control fish group which showed 0.16 g./fish. The same trend was observed in the result of specific growth rate (SGR) as showen in Table (2), where (T_4) in small size had the optimum value (1.95 %) while the lowest value (1.50/0), was recorded from the control. S.G.R in big size fish T_4 had a significant differences among all treatments ($p<0.05$), it showed the highest significant value of 1.33%, while the control groups T_1 recorded the lowest value (0.44%), the survival percent of the tested fish had significant differences among the treatments group ($p<0.05$), in small size fish T_4 had a highest value (96%), while T_1 , showed the lowest value 82%, in case of big sized fish also the same trend T_4 had a significant highest value (97%) and T_1 recorded the lowest value (80%), Table (2) shows the results of feed conversion rate (FCR) as it has a significant difference ($p<0.05$) among the treatment groups. T_4 which feed diets contained the highest level concentration of *B. subtilis* with the best and lowest F.C.R value (1.7 g.) feed for each g. gain in weight in small sized fish, the worst value (2.0) was recorded from the control T_1 and the differences among the treatments FCR were significant ($p<0.05$).

Table (2) Average final body weight, aver. daily gain, survival ratio, feed conversion ratio and specific growth rate for *O. niloticus* fingerlings fed diets contained *B. subtilis* as a probiotic. and growth promoter

Treatments Items	**T_1**	**T_2**	**T_3**	**T_4**	**T_1**	**T_2**	**T_3**	**T_4**
Av. Initial body weight g./ fish	$3.5^{\pm 0,02}$	$3.5^{\pm 0,02}$	$3.5^{\pm 0,02}$	$3.5^{\pm 0,02}$	$15.5^{\pm 0.02}$	$15.5^{\pm 0.02}$	$15.5^{\pm 0.02}$	$15.5^{\pm 0.02}$
A v. final body weight	$18.7^{+0.03}$	$20.2^{+0.02}$	$22.5^{+0.03\ ab}$	$28.5^{+0.05}$	$23.0^{+0.02c}$	$46.3^{\pm 0.06\ ab}$	$55.6^{\pm 0.03}$	$80.5^{\pm 0.03}$
Av. Weight gain g / fish	$15.2^{\pm 0,05\ c}$	$16.7^{\pm 0.01\ b}$	$19.0^{+0.02\ ab}$	$25.0^{\pm 0,03\ a}$	$17+^{0.05\ c}$	$31.2^{\pm 0.03\ ab}$	$40.2^{+0.02\ b}$	$65.0^{+0.02\ a}$
Daily weight gain g/fish day.	0.16^{c}	0.18^{b}	0.21^{ab}	0.27^{a}	0.18^{c}	0.34^{ab}	0.44^{b}	0.71^{a}
S.GR%	1.5^{c}	1.6^{b}	1.72^{ab}	1.95^{a}	0.44^{c}	0.86^{ab}	0.96^{b}	1.33^{a}
Survival	80^{c}	82^{b}	90^{ab}	95^{a}	80^{c}	86^{ab}	90^{b}	97^{a}
Feed conversion ratio.	2.00^{c}	1.90^{b}	$1.8^{\ ab}$	$1.70^{\ a}$	$2.1^{\ c}$	1.9^{ab}	1.7^{b}	1.6^{a}

$SE^{\pm}$ is the standard error calculated from residual mean in the analysis or variance.

a,b,c... etc means in the same row the different superscript are significantly different ($p<0.01$).

Serum biochemistry

Total protein (TP) content, (A) Albumin, (G) globulin and A:G ratio are presented in Table (3). Total protein in small size fish had significant differences T_2, T_3 and T_4 recording values of ($2.37^{+0.05}$, $2.481^{+0.04}$, and $2.481^{+0.01}$ respect.). They increased by increasing the concentration of *B. subtilis* with respect to

control, its recorded value (2.311) the big sized fish showed $2.496^{+0.03}$ $2.587^{+0.01}$ and $2.886^{+0.03}$ respectively) compared to control; it recorded ($2.416^{+0.02}$) and the changes were not significant. Albumin content as shown from Table (3), all the treatments in small size fish T_2, T_3 and T_4 values ($1.311^{+0.04}$, $1.231^{+0.02}$, and 1.213) fish offered diets contained *B. subtilis* showed a significantly higher value ($P<0.05$) of albumin than the control T_1 ($1.321^{\pm 0.06}$), big fish groups (T_2, T_3 and T_4) records were, ($1.41^{\pm 0.0,1}$, $1.332^{\pm 0,6}$) and $1.418^{\pm 003}$) had a significant higher ($p<0.05$) value than the control groups. T_1 (1.428), Globulin content shown in Table (3) all treatments group T_2, T_3 and T_4 recorded values of $1.295^{\pm 1.321}$, $1.321^{\pm 0.01}$ and $1.398^{\pm 0.0}$, which had diets containing *B. subtilis* had a significant high levels of globulin than the control group in small sized fish. Also, it has shown the same trend in big fish group in T_2 , T_3 and T_4 that had significantly higher values ($p<0.05$) than those in control T_1 , which recorded values of ($1.382^{\pm 0.06}$ $1.42^{\pm 0.03}$ and $1.487^{\pm 0.03}$ with the respect. to T_1 ($1.283^{\pm 0.02}$).

Albumin-Globulin rates as illustrated in Table (3), difference was significantly higher ($p<0.05$) in T_1 ($1.234^{+0.01}$) and significantly lower in T_4 (0.87) in small size fish, but in big size fish, this ratio recorded the highest value ($p<0.05$) in T_1 ($1.113^{+0.03}$) and significantly lower in T_4 ($0.882^{+0.03}$).

Table (3) Effect of adding *B. subtilis* to tilapia fingerling feeding on total protein albumin, globulin contents and the albumin- globulin ratio.

Treatments / Items	Small size fish				Big size fish			
	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
	$\pm$ SE	$\pm$ SE	$\pm$ SE	$\pm$ SE	$\pm$ SE	$\pm$ SE	$\pm$ SE	$\pm$ SE
TP (g-dL^{-1})	$2.311^{\pm 0.06}$ c	$2.341^{\pm 0.01}$ ab	$2.481^{\pm 0.01}$ b	$2.481^{\pm 0.01}$ a	$2.416^{\pm 0.03}$ c	$2.496^{\pm 0.03}$ ab	$2.587^{\pm 0.01}$ a	$2.80^{\pm 0.03}$ a
(A) Albumin (g-dL^{-1})	$1.321^{\pm 0.05}$ c	$1.311^{\pm 0.03}$ ab	$1.231^{\pm 0.02}$ b	$1.231^{\pm 0.06}$ a	$1.428^{\pm 0.03}$ c	$1.411^{\pm 0.02}$ ab	$1.332^{\pm 0.06}$ b	$1.418^{\pm 0.03}$ a
(G) Globuline (g-dL^{-1})	$1.070^{\pm 0.03}$ c	$1.295^{\pm 0.03}$ ab	$1.328^{\pm 0.03}$ b	$1.398^{\pm 0.01}$ a	$1.283^{\pm 0.02}$ c	$1.382^{\pm 0.06}$ ab	$1.423^{\pm 0.02}$ b	$1.487^{\pm 0.02}$ a
A- G Ratio	$1.234^{\pm 0.01}$ c	$1.011^{\pm 0.01}$ ab	$0.926^{\pm 0.03}$ b	$0.87^{\pm 0.03}$ a	$1.113^{\pm 0.03}$ c	$1.020^{\pm 0,03}$ ab	$0.949^{+0.01}$ b	$0.882^{\pm 0.02}$ a

The Tp (g-dL^{-1}) = Total protein, gram / distilled liter.

mean values in row and column the same superscripts for individual parameter do not vary significantly ($P<0.05$).

$SE^{\pm}$ is the standard error calculated from residual mean in the analysis or variance.

a,b,c... etc means in the same row the different superscript are significant different ($p<0.0l$).

DISCUSSION

The growth performance parameters such as final body weight (FBW), total weight gain (TWG), average daily gain (AD G) and specific growth rate (SGR) of Nile tilapia fingerlings as affected by adding *B. subtilis* at three concentrations (5,10 and 15 ml solution) contained 10^8 CFU ml^{-1} to the diet was more pronounced for the three levels of *B. subtilis* .The results showed that they had the highest value in both final body weight, daily weight gain for fish grop fed diet contained *B. subtilis* for fish of both small size and big size groups. These results are in agreement with results of Abd EI Hakim *et al*. (2006), who revealed that, using of the probiotic Biogen® at level 3.0 g./ kg diet showed the best results on growth performance parameter of Nile tilapia fry. Also these results are in accordance with the findings of Jena *et at*., (1999), who indicated that Catla and Rohu Fry fed on experimental diets with probiotic supplement had a significant increase in length and weight at different fish stages of the experiment compared with those fed on control diet.

Including probiotic in fish diets improved fish growth rate and consequently the final body weight in most studies carried out (Thompson *et al*., 1999; Dpuil fet, 2000; Venkat *et al*., 2004).

Results of S.G.R. showed that the supplemented diet of small size Nile tilapia as affected by adding *B. subtilis*, T_4 gave higher value and recorded significant increases in S.G.R, with contrast to the control group, which recorded the lowest value. In comparative studies with the results of S.G.R. for the big size and small size groups, it was clarified that T_2 , T_3 and T_4 in small fish groups had significant increases in S.G.R. than those in big fish groups. These results contrast to these of Abo-State, (2005) who showed that supplementary diet of Nile tilapia fry (1.0 g initial weight) with lecture, Yeast or Premalace, probiotics, 1 , 2 or 3 g/kg diet did not improve body weight gain and (SGR) of fish compared to the control.

Survival rates in this study achieved a significant increase in survival in treatment groups T_2, T_3 and T_4 with contrast to the control, These results are in accordance with Reitan *et al.* (1997), who indicated that addition of micro algae to the first feeding tanks along with rotifers improved growth and survival of larvae.

Serum parameters showed a significant increases in total protein content, serum Albumin with best increase in the treatment fish groups offered diet contained *B. subtilis* than those offered the control diet. Also globulin recorded the highest value in the treatment fish groups fed the tested diet which contained *B. subtilis* with contrast to the control, both in small and big size fish. These results are in accordance with that of Balcàzar *et al*. (2006), who showed that increasing in serum protein and globulin levels was thought to be associated with a strong innate response in fish.

Wiegerties *et al.* (1996), showed that the ratio between Albumin-globulin was decreased in fish groups offered diets that contained 15 ml.

solution of *B. subtilis* followed by increasing in the other treatment fish groups. These results mean that the decrease in A: G. ratio in high level contents of adding *B. subtilis* concentration to the diet is indicative of better immunity of the animal which may occur due to an increase in the animal globulin level compared with albumin.

The increase in total serum protein and globulin indicates that fish are immunologically strong, as shown by Nayak *et al*. (2004).

Comparative study parameters such as growth performance including FBW,WG,ADG,SGR, feed conversion and serum chemical parameters studies between the .small sized and big. size fish groups of Nile tilapia fingerlings fed on commercial supplementary diet with adding *B. subtilis* at three different levels. 5.10, and 15 ml contained 10^8 CFU ml^{-1} /100g diet show that small size fish groups had the highest recorded values and _the best results. They were , more benefited from addition of probiotic than the big size fish groups.

So it can be concluded that the observed improvement in fish immune parameters as well as growth and survival, using probiotic bacteria may open a new scope for intensive use in aquaculture.

The results recorded that *B. subtilis* used in this research can be used effectively as a commercial product for use in aquaculture.

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