Effect of prebiotic on the immune status of *Oreochromis niloticus*

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A B S T R A C T

A total number of 180 Nile-tilapia *(Oreochromis niloticus)* were divided into four equal groups; the first one served as control fed on basal non-treated diet. The second group fed on diet supplemented with 0.5 ml prebiotic βpolo /kg basal fish diet, the third group on diet supplemented with 1 ml prebiotic βpolo/kg basal fish diet, and the fourth group fed on diet supplemented with 1.5 ml prebiotic β polo/kg basal fish diet for 40 days. The result elevated that, the growth performance was significantly higher in all treatments than in the control group and the highest increased was in the group received 1.5 ml prebiotic βpolo /kg basal fish diet. The hematocrit (HC) values, nitroblue-tetrazolium (NBT) and lysozyme activities were increased significantly in all treated groups than the control and the highest increased was in the group received 1.5 ml prebiotic βpolo /kg basal fish diet. The percent level of protection among the three treated groups after challenge infection using *Pseudomonas flourescence* (*Ps. flourescence*) (0.5 ml of culture suspension of pathogen containing 10^7 bacteria ml^{-1}) was higher than control and the highest record was in the group of received 1.5 ml prebiotic βpolo /kg basal fish diet .

Key words: Prebiotic, immune response, *Ps. flourescence, Nile tilapia.*

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1. INTRODUCTION

During the last three decades a great attention was paid towards aquaculture as an attempt to fulfill the gap between the increased population and their demand from animal protein. Aquaculture is still the fastest growing food producing sector, compared to other food commodities with an annual increase of approximately 12% (FAO, 2004 and FAO, 2009). Nile tilapia, *Oreochromis niloticus* (L.) is an important species for freshwater aquaculture and the most widely cultured in tropical and subtropical countries. Consumers like tilapia's firm flesh and mild flavor, so markets have expanded rapidly (FAO, 2005). Bacteria were introduced to the fish from through natural or artificial feed sources, treated inlet water and less frequently through vertical transmission from brood's tock (Sandaa et al., 2003 and Schulze et al., 2006). Bacteria constitute the most economically significant group of pathogenic agents. Bacterial diseases were responsible for heavy mortalities in fishes throughout the world and the most of causative microorganisms were naturally occurring opportunist pathogens which invade the tissue of a fish host rendered susceptible to infection (Roberts et al. 2000). The use of antibiotics in aquaculture as disease

prevention and growth promotion may introduce potential hazard to public health and to the environment by the emergence of drug-resistant microorganisms and antibiotic residues killed or inhibited by oral chemotherapy (Sugita et al., 1991 and FAO/WH O/OIE 2006). The use of prebiotics in aquaculture is now widely used instead of chemotherapy and antibiotics to increase safety protein production for human. New strategies in feeding and health management in fish aquaculture practice had received much attention (Balcazar et al*.,* 2006); in developing new dietary supplementation strategies in which various health- and growth-promoting compounds as probiotics, prebiotics, synbiotics, phytobiotics and other functional dietary supplements had been evaluated (Denev, 2008). The most promising group of immune-stimulants are the 1,3/1,6-glucans, because they have a well-defined chemical structure and mode of action on the immune system. In addition, 1,3/1,6-glucans are non-toxic universal "alarm signals" which activate the immune system by the same basic mechanism in all animal groups, from the simplest invertebrates to man. 1,3/1,6-Glucans are active not only when injected, but also when administered in the feed, or on mucosal surfaces. The *b-1,3-glucans* found in mycelial fungi and yeast, differ from immune-stimulants of bacterial origin in chemical structure and mode of action. Moreover, it has been shown that 1,3-glucans may improve health, growth and general performance of many different animal groups, including farmed shrimp, fish and land animals ("*health promoting food"*) sector (Raa, J.2000). The objectives of the current investigation were to evaluate the potential of prebiotics for optimizing growth performance and increasing immunity of Nile tilapia against *Ps. flourescence*.

2. MATERIALS AND METHODS

2.1. Fish

Apparently healthy Nile tilapia (*Oreochromus niloticus*) of 30±5 g/fish weight and 10±2cm length were collected from the ponds of the Central Laboratory for Aquaculture Research. Total number of 180 fish were kept in previously prepared 12 glass aquaria (70×60×50 cm) for 40 days. The aquaria were supplied with dechlorinated f water. The average of the temperature was 25°C ± 1°C and the oxygen was adjusted for continuous aeration by using electrical air pumping compressors (RINA, Italy). Fish were acclimated under the laboratory conditions in indoor tanks for 2 weeks.

2.2. Prebiotics:

Product name: β-polo (prebiotic), Composition: each 1liter contains: β-glucan 30ml, Propylene glycol 1.0ml, Purified water up to 1000ml in Package: 1liter.Company: Naturance Imported by: Sky Pharma.

2.3. Experimental diets:

Fish were divided into four groups; each group, in three replicates. Each group contained 15 fish. The first group (T1) fed with control diet (basal non-treated diet). The second group (T2) was treated with 0.5 ml prebiotic βpolo /kg standard fish diets). While, the third group (T3) was treated with 1.0 ml prebiotic βpolo /kg standard fish diets. The fourth group (T4) was treated with 1.5 ml prebiotic βpolo /kg standard fish diets /kg. The fish fed three times per day for 40 days at a daily rate 3% of body weight. The water of the aquaria was changed daily. The fish were weighted after 10, 20, 30 and 40weeks from the beginning of the feeding experiment

2.4. Growth performance:

The average weight-gain (AWG), specific growth rate (SGR), feed conversion ratio (FCR) and feed efficiency ratio (FER) were calculated according to (Tekinay and Davis, 2001) through the following equations: AWG (g/fish) = Average final weight (g) – Average initial weight (g)/ experimental period (day).SGR (%/day) = 100 [ln final body weight (g) – ln initial body weight (g)]/experimental period (day).FCR (%) = Feed intake (g) / weight gain (g).FER (%) = Body weight gain (g) / Feed intake (g).

2.5. Blood and serum sampling:

Blood samples were collected before and after feeding experiment (every 10 days for 40 days) from the blood vessels of fish, by using EDTA coated syringes (15unit/ml) for the evaluation of hematocrit value and respiratory burst activity. For plasma separation the blood with EDTA was centrifuged at 3000 rpm and 4°C for 5 minutes. The supernatant was stored at -20°C in screw cap glass vials until used for lysozyme activity estimation.

2.6. Hematocrit level:

Hematocrit capillary tubes are filled 2/3 with heparinized whole blood, tube were centrifuged in hematocrit centrifuge for 5 minutes. After centrifugation, the percentage of erythrocyte volume was measured by hematocrit tube reader (Smith, 1967).

2.7. Respiratory burst activity using nitro bluetetra-zolium activity (NBT):

0.1 ml blood was placed into micro titer plate then equal amount of 0.2% NBT solution was added and incubated for 30 min at room temperature, 0.1 ml of NBT blood cell suspension was taken and added to a glass tube containing 1 ml N, N-dimethyl formamide and centrifuged for 5 min at 3000 rpm, the Respiratory burst activity was read in spectrophotometer at 620 nm in 1 ml cuvettes (Siwickiet al., 1985).

2.8. Lysozyme activity:

The lysozyme activity was measured by using electric colorimeter with attachment for turbidity measurement. A series of dilution was prepared by diluting the standard lysozyme from hen egg-white (Fluka, Switzerland) and mixed with *Micrococcus lysodeikticus* (ATCC NO. 1698 Sigma) suspension for establishing the calibration curve. Ten µl of standard solution or serum were added to 200 µl of *Micrococcus* suspension (35 mg of *Micrococcus* dry powder/95 ml of 1/15 M phosphate buffer 0.5 ml of NaCl solution). The changes in the extinction were measured at 546 nm by measuring the extinction immediately after adding the solution which contained the lysozyme (start of the reaction) and after 20 min incubation of the preparation under investigation at 40°C (end of the reaction). The lysozyme content was determined on the basis of the calibration curve. The extinction was measured (Schaperclaus et al., 1992) using spectrophotometer at 620 nm in 1 ml cuvettes (Siwicki et al., 1985).

2.9. Microbial strains:

Pseudomonas flourescence (*Ps. Fluorescence)* for challenge test was kindly supplied by fish Health and management department, Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharkia Egypt.

2.10. Challenge test:

The treated groups as well as the control groups were challenged by I/P injection of 0.5 ml 10^7 cells of 24 h cultures of virulent *Ps. Fluorescence* at the ends of 40 days' post feed experiment. Fish were observation for two weeks post challenge for

mortalities rate. Blood samples were collected at the end of the 4o days post feed experiment. serum separated, inactivated at 56C for 30 minutes then kept at -2C till being used, and the relative level of protection (RLP) calculated according to the equation of (Newan and Majnarichsm1982) RLP =1 -[percentage of treated mortality/ percentage of control mortality] x100.

2.11. Determination of Antibody titer:

Antibody titer in collected sera were determined using indirect hematoagglutination method the antibody titer of T1,T2,T3 and T4 was measured using indirect hematoagglutination method after challenge test by bacterium strain *ps.fluresence* according to (Schaperclaus et al. 1992).

2.12. Statistical analysis

Statistical analysis was performed using the one-way analysis of variance (ANOVA) Murray (1975). It was performed with SPSS statistical software (version 10.0, SPSS). The data were subjected for test of homogeneity of variances and Duncan post-hoc test. Data were considered significantly different when $P < 0.05$

3. RESULT

3.1. Growth performance:

The (AWG) showed a significant increase in T_4 compared to T1 and no significant change in T2 andT3 compared to control. The specific growth rate (SGR) obtained significant increase in T2, T3 and T4 in comparison to control. Also, feed conversion ratio (FCR) of *O. niloticus* showed significant decreased inT2, T3 and T4. Compared to control. The best (FER) values showed significant increase inT4 compared to control. The AWG, SGR and FCR showed significant increase in T4(the highest value in T4 compared to control) and in FER obtained significant decrease in T4compared to control ($P < 0.05$) (Table 1).

3.2. Heamatocrite value:

Hematocrit value after 10 days showed no significant difference in all treated groups T2, T3 and T4 in comparison with T_1. After 20 days, 30 days and 40 days, HC showed significant increase in T3 and T4 in comparison with T_1 as shown in (Table2). The highest value of HC obtained at T4(63.33±0.33) after 40 days in comparison with T_1 and the lowest value of HC obtained at T2 (27.33±0.22) after10 days in comparison with T_1 (Table2).

3.3. Respiratory burst activity by measuring nitro blue titrazolium activity (NBT)

After 10 days and 20 days, NBT assay showed significant increase in T2, T3 and T4 in comparison with T_1. After 30 days and 40 days, NBT assay showed significant increase in T3 and T4 in comparison with T_1 and showed no significant change in T2 in comparison with T_1 as shown in (Table 3). The highest value of NBT assay obtained at T4 (3.95±0.217) after 40 days in comparison with T_1 and the lowest value of NBT assay obtained at T2 (1.05±0.145) after 30 days in comparison with T_1 (Table 3).

3.4. Lysozyme activity:

The result in table (4) illustrated the value of lysozyme activity showed significant increase all over the experiment period inT2, T3 and T4 compared to control group. The highest value of lysozyme obtained at T4 (3.05±0.217) after 40 days in comparison with T_1 (control) and the lowest value of lysozyme obtained at T2 (1.21±0.23) after 30 days in comparison with T_1(Table 4).

3.5. Challenge test:

Table (5) it was noticed that, the mortality percentage showed higher mortality with T_1 (53.33) and the lower mortality observed in T4. Relative level of protection was higher in T4 (100%) as in Table (5).

3.6. Antibody titer:

Results of table (6) showed that the highest level of the antibody titre to *Ps. flourescence* infection was obtained with T_4 (9 log10). T_2, T_3 Compared to T1.

Table (1): The effect of three different doses of prebiotic β polo supplemented diet on growth parameters in *O. niloticus* fed for forty days.

Parameter	Treatments			
	T_1	T_2	T_3	T_4
AWG	0.15 ± 0.01^{b}	0.26 ± 0.04^{b}	0.33 ± 0.05^{b}	0.52 ± 0.08^{a}
SGR	0.69 ± 0.03^{b}	1.04 ± 0.02^{a}	1.09 ± 0.08^{a}	1.05 ± 0.43^{a}
FCR	0.53 ± 0.01^{b}	0.60 ± 0.03^{b}	0.77 ± 0.06^{a}	0.97 ± 0.12^{a}
FER	1.10 ± 0.16^{b}	1.87 ± 0.05^{a}	1.67 ± 0.15^{a}	1.31 ± 0.12^{b}

Means carrying different superscripts are at ($p\leq 0.05$). (a- c) increasing significant in the same

Table (2): Effect of prebiotic βpolo supplemented diet on hematocrit value in *O. niloticus* for forty days of feeding experiment.

Treatments	10 days	20 days	30 days	40 days
T1	1.02 ± 0.09^{bc}	1.08 ± 0.08^{b}	1.07 ± 0.09^{b}	1.40 ± 0.18^{b}
T2	2.15 ± 0.20^{a}	1.50 ± 0.09^{a}	1.05 ± 0.14^{b}	1.26 ± 0.21^{b}
T3	2.29 ± 0.09^{a}	1.96 ± 0.39^{a}	2.84 ± 0.05^{a}	2.99 ± 0.42^{a}
T4	2.30 ± 0.36^{a}	2.18 ± 0.13^{a}	3.03 ± 0.10^{a}	3.95 ± 0.21^{a}

Means carrying different superscripts are at ($p\leq 0.05$). The values were given as means (±S.E.) of three replicates. Means with different letters within column differ significantly, $P \leq 0.05$, Means with same letters within column non-differ significantly, $P \leq 0.05$ (a- c).

Table (3): Respiratory burst by using (NBT) assay in *O. niloticus* after feeding prebiotic βpolo for forty days.

Treatment	10 days	20 days	30 days	40 days
T1	1.26 ± 0.99^{b}	1.27 ± 0.18^{b}	1.41 ± 0.19^{b}	1.63 ± 0.12^{b}
T2	1.25 ± 0.20^{b}	1.22 ± 0.42^{b}	1.21 ± 0.23^{b}	1.42 ± 0.25^{b}
T3	1.76 ± 0.59^{a}	1.71 ± 0.21^{a}	2.71 ± 0.09^{a}	2.97 ± 0.26^{a}
T4	2.06 ± 0.36^{a}	1.99 ± 0.21^{a}	2.82 ± 0.36^{a}	3.05 ± 0.21^{a}

Table (4): Serum lysozyme activity in *O. niloticus* after prebiotic βpolo supplemented diet for forty days.

Treatments	10 days	20 days	30 days	40 days
T1	35.00±0.00[a]	26.66±0.80[b]	32.33±1.45[b]	31.33±0.02[b]
T2	27.33±0.22[a]	32.33±1.45[b]	34.00±0.05[b]	43.33±0.06[b]
T3	40.00±0.77[a]	46.66±0.80[a]	47.33±0.88[a]	56.00±0.50[a]
T4	43.30±0.66[a]	51.66±0.40[a]	56.66±0.81[a]	63.33±0.33[a]

Table (5): Mortality rate and Relative level of protection in treated *O. niloticus* after challenge with *Pseudomonas flourescence*.

treatments	*Pseudomonaus flourescence*	
	Mortality%	RLP
T_1	53.33	0
T_2	20	62
T_3	10	81
T_4	5	90

Table (6): Effect of prebiotic βpolo supplemented diet on the antibody titre level due to *Ps. Fluorescens* infection.

Antigen	Antibody titre (log_{10})			
	T_1	T_2	T_3	T_4
Ps. flourescence	4	5	7	9

4. DISCUSION

The statistical analysis of different growth parameters of *O. niloticus* in (table 1) at the end of feeding period the (AWG) showed a significant increase in T_4 compared to T1 and no significant change in T2 andT3 compared to control. The specific growth rate (SGR) obtained significant increase in T2, T3 and T4 in comparison to control. Also, feed conversion ratio (FCR) of *O. niloticus* showed significant decreased inT2, T3 and T4. Compared to control. The best (FER) values showed significant increase in T4 compared to control. The AWG, SGR and FCR showed significant increase in T4(the

highest value in T4 compared to control) and in FER obtained significant decrease in T4compared to control. And this agree with (Rudabeh Rufchaie and Seyed Hossein Hoseinifar,2014) reported that the growth parameters such as final body weight, weight gain, SGR and FCR were improved by the inclusion of a low dose (0.5% and 1%) dietary CYG. Similar benefits have been reported in fish fed dietary glucan (Aramli MS et al 2015). The result of (table2) obtained the hematocrit value showed a significant increase for T_3 and T_4 all over the experiment period in comparison with T_1, T2 showed no significant change compared to T1. It could be noticed that there was significant difference between treated groups. Our study supported by and (Jamal et al. 2014) and (Abd El-Rhman, 2009). The result of (table 3) obtained, NBT values obtained significant increase in T_3 and T_4 all over the experiment in comparison with T_1. NBT values showed significant increase in T2 after 10 and 20 days compared to T1. The result obtained no significant change compared to T1 after30 and 40 days The present study agree with Ai et al. (2007) showed that Low concentration of glucan (0.09%) significantly enhanced the respiratory burst and phagocytic activity in head kidney macrophages and agree with (He et al., 2011). The result of (table 4) obtained lysozyme activity increased significantly all experiment period in T_3 and T_4 but no significant difference in T_2 in comparison with T_1. The increased lysozyme activity has been reported after supplementing with β-glucan agree with El-Boshy et al., 2010 who reported that All dietary CYG levels significantly increased the innate immune responses (lysozyme activity). And agree with Seyed Hossein Hoseinifar (2014) and Aramli MS et al. (2015).The result of (table 5) mortality rate and relative level of protection of *o.niloticus* after i/p with pathogenic *Pseudomonas fluorescence* agree with Ai, Q., K. (2007) who reported that multiple injections of -glucan enhance the immune response and disease resistance against opportunistic pathogens *Aeromonas hydrophila & Edwardsiellata and agree with .also supported by* The β-1,3-glucans of certain yeasts have been successfully used as immune-stimulants to enhance the defense potential of fish and shellfish against bacterial and viral infection and agree with (Abdel-Tawwab et al., 2008) In (table 6) antibody titer showed that the highest level of the antibody titer after i/p with *Pseudomonas fluorescence* was obtained with T_4 and the lowest value T_2 compared with control and this result agree with Sirimanapong W et al(2015).

5. CONCLUSION

Prebiotic β-polo at 1.5 and 1 ml/kg fish diets improve growth performance and immune response of Nile tilapia *(Oreochromis niloticus*) by increasing respiratory brustactivity, lysozyme and hematocrit level and also, increase resistance against *Pseudomonas flourescence*.

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