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Effects of probiotic and prebiotic diet supplements on growth performance, immune response and disease resistance of juvenile Nile tilapia *Oreochromis niloticus* (L.)

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Abstract

This study was carried out to investigate the effects of probiotic, *Saccharomyces cerevisiae* and/or prebiotic, Mannan-oligosaccharides MOS, as diet supplements on growth performance, body composition, immune response and disease resistance of juvenile Nile tilapia *Oreochromisniloticus* (L.). A total of 540 of Nile tilapia fingerlings (weight ranged from 32.22 to 37.08 g) were randomly allotted into nine treatment groups (T_1:T_9) with three replicates per each. The probiotic, *S. cerevisiae* and/or prebiotic, MOS were included at levels of 0.0/0.0 (control, T_1), 0.5/0.0, 1.0/0.0, 0.0/0.5,0.5/0.5, 1/0.5, 0.0/1, 0.5/1 and 1/1 g/kgdiet; respectively; for 12 weeks. By the end of experiment, fish of each treatment were challenged by pathogenic *Aeromonashydrophila*, given by intraperitoneal (IP) injection and kept under observation for 7 days to record clinical signs and the daily mortality rate. The growth-promoting influences of *S. cerevisiae* and/or MOS were observed in fish. Body weight development was improved in all *S. cerevisiae* and/or MOS supplemented groups. Fish of T_9showed a significant increase in body weight gain (WG), body weight gain percentages (BWG %), specific growth rates (SPG %), feed conversion ratio (FCR) and protein efficiency ratio (PER) versus the control group. In addition, *S. cerevisiae* and/or MOS supplementation increased protein deposition in fish body of T_9. The results of hematological parameters and immune response revealed that, hemoglobin (Hb), hematocrit (HT %) and phagocytic activity was also improved in all *S. cerevisiae* and/or MOS supplemented treatments. Improvements due to T2 and T_7 were statistically significant. On the other hand, lymphocyte significantly increased in fish of T_5 and T_9, while monocyte count was significantly higher in fish of T_6, meanwhile, the leukocytic count was not affected with any supplementation. The serum total protein, albumin and A/G ratio significantly increased in fish of T_5, T_6, T_8 and T_9 when compared with control group. Higher antibody titers against *A. hydrophila* infectionand lower mortality percentages were observed in T_3, T_6, and T_9 fish groups, seven days, post infection. Similarly, the highest value (75.1%) of relative level of protection (RLP%) observed in T_3, T_6, and T_9 fish groups. Accordingly, these results indicated that,the use of probiotic (*S. cerevisiae*) and prebiotic (MOS) combination as a diet supplement had a synergistic effect in enhancing growth performance and feed utilization of Nile tilapia as well as its resistance to *A. hydrophila* infection.

Keywords: Probiotic, Prebiotic, *Oreochromisniloticus*, Growth performance, Disease resistance

1. Introduction

World aquaculture has grown tremendously during the last years and becoming an economically important industry (Subasinghe *et al*., 2009). Today it is the fastest growing food-producing sector in the world with the greatest potential to meet the growing demand for aquatic food (FAO, 2009). Globally, aquaculture is expanding into new directions, intensifying and diversifying. A persistent goal of global aquaculture is to maximize the efficiency of production to optimize profitability.

During the last decades, antibiotics were not only used as traditional strategy for fish diseases management, but also for the improvement of growth and efficiency of feed conversion. However, the development and spread of antibiotics resistant pathogens were well documented (SCAN, 2003; Kim *et al*., 2004; Cabello, 2006; Sorum, 2006). On the other hand, antibiotics inhibit or kill beneficial microbiota in the gastrointestinal (GI) ecosystem with accumulated antibiotic residue in fish products (WHO, 2006). So, since January, 2006 European Union ratified a ban for the use of all sub-therapeutic antibiotics as growth-promoting agents in animal production.

In connection with the ban of antibiotic growth promoters (AGP) new strategies in feeding and health

management in fish aquaculture practices have received much attention (Balcázar *et al*., 2006). In addition, the global demand for safe food has prompted the search for natural alternative growth promoters to be used in aquatic feeds. There has been heightened research in developing new dietary supplementation strategies in which various health and growth-promoting compounds as probiotics, prebiotics, synbiotics, phytobiotics and other functional dietary supplements have been evaluated (Denev, 2008).

In recent years, many published reports demonstrated positive effects of probiotics and prebiotics in feed for various fish species, including Nile tilapia (*Oreochromis niloticus*) Francis *et al., 2005; EL-Harounet al., 2006; Taokaet al., 2006; Abdel- Tawwabet al., 2008; Marzouk et al., 2008; Pirarat et al., 2008;Ricardo et al., 2008; Ali et al., 2010;Eid et al., 2010; Ibrahim MD, et al., 2010; Lara-Flores et al.., 2010; Schwarz et al., 2010)*, Mozambique tilapia (*Oreochromismos sambicus*; *Logambal et al., 2000*), hybrid tilapia*(Oreochromis aureus*♂×*O. niloticus*♀; *Hui-yuan et al.,* 2007).

Although application of pro- and prebiotics as environment-friendly alternatives of AGP in fish nutrition seems to be relatively recent, the interest in such dietary supplements is increasing rapidly not only in fish, but also in Shrimp aquaculture (Dalmo and Bøgwald, 2008; Wang *et al.,* 2008; Zhang *et al.,* 2009).

In North Africa, Egypt is by far the dominant country in terms of aquaculture production (99 percent of the regional total) and, in fact, is now the second largest producer of tilapia after China and the world's top producer of mullets (FAO, 2009).The current trend in tilapia farming in Egypt is towards increased intensification of culture systems. Success in this type of culture is highly dependent on the availability of well balanced, nutritionally complete and cost-effective formulated feeds. On this context, many probiotics and to some extent prebiotics are successfully used for growth and health management in the sustainable aquaculture industry in Egypt. The main advantage of prebiotics over probiotics is that they are natural feed ingredients. Their incorporation in the diet does not require particular precautions, and their authorization as feed additives may be more easily obtained, in spite of some concerns about their safety and efficacy. Although scientists cited above have demonstrated beneficial effects of using probiotics or sometimes prebiotics as fish feed additives, information on the interaction among prebiotic spices, digestive enzymes and probiotics in fish diets is very scarce.

Therefore the objectives of this study were to investigate the effect of two commercial probiotics, Tonolast® (*Saccharomyces cerevisiae*) and/or Prebiotics, AG-FLO®, (Mannanoligosaccharides, MOS) on growth performance, body composition, as well as the immunity of Nile tilapia fingerlings (*Oreochromis niloticus*). Their possible protective effect against an experimental challenge infection using *A. hydrophila* has been also investigated.

2. Materials and methods

2.1. Experimental fish and housing facilities

A total of 600 apparently healthy Nile tilapia (*O. niloticus*) fingerlings were obtained from a private fish hatchery in Behaira Governorate, Egypt. They were transported to the fish breeding and production laboratory belongs to the Department of Animal Husbandry and Animal Wealth Development, Faculty of Veterinary Medicine, Alexandria University.

A total of 540 and fingerlings were randomly and equally divided into nine treatments with triple replicates and carefully stocked into glass aquaria (85 x 40 x 65 cm), as 20 *O. niloticus* in each aquarium. A further 60 fingerlings were kept in three other aquaria to compensate any dead fish during the adaptation period. Fish were adapted to the experimental conditions for a couple of weeks for adaptation, and fed the basal diet without any additives at a ratio of 3% of body weight per day. Natural light was available providing nearly 12 hrs light/day (Meske, 1985). Each aquarium was cleaned every morning prior for feeding by siphoning the wastes which had accumulated on the bottom, replaced by aerated water thorough dechlorinated tap. Water samples were taken every week and the following physico-chemical parameters of water; water temperature (27.0±0.1)°C, DO (6.50±0.20) mg O_2/L, pH (7.33±0.04), NH_3-N (0.35±0.02) mg N/L. were recorded. Water temperature was recorded in degrees centigrade using a mercury thermometer. NH_3-N was analyzed using analytical kits (HACH Company, Loveland, CO 80539 USA). The pH value using electric digital pH meter Orion Research model 201and dissolved oxygen was determined by using the azide modification method after the American Public Health Association (APHA, 1992).

2.2. Experimental diets

Pellets (2mm) were prepared from locally available ingredients (Tables 1, 2). They were finely ground and thoroughly mixed with the used vitamins and minerals, then the diet divided into nine groups, each group was supplemented with each specific levels of probiotics (Pro), and/or prebiotics (Pre), at levels of 0.0/0.0 (control), 0.5/0.0, 1.0/0.0, 0.0/0.5,0.5/0.5, 1/0.5, 0.0/1, 0.5/1 and 1/1 g /kg diet. The ingredients were blended with additional 100 ml of water per 1 kg diet to make a paste of each diet. The pastes were passed through a grinder and pelleted (2 mm diameter) in a paste extruder. The obtained pellets were allowed to air dry at room temperature for 24 h. The required diet was prepared biweekly and stored in a refrigerator (4°C) for daily use.

Table 1. Ingredients composition (%) of the basal diet

Ingredients	%
Fish meal (72%)	27
Soya bean meal (44%)	23
Ground yellow corn	35
Wheat bran	10
Binders *	2
Mineral mix**	1.5
Vitamin mix#	1.5

*Binders: Sodium carboxy methyl cellulose (high viscosity) according to Murai *et al*., (1986).
**Mineral mix according to *Jauncy and Ross (1982)* and it contains the following (g/kg): Magnesium sulphate, 255.0000; Sodium chloride, 120.0000; Potassium chloride, 100.0000; Iron sulphate, 50.0000; Zinc sulphate, 11.0000; Manganese sulphate, 5.0750; Copper sulphate, 1.5700; Cobalt chloride, 0.8080; Potassium iodate, 0.5005; Chromic chloride, 0.2550. These minerals were weighed and made up to 1 kg using starch as bulky agent.
#Vitamin mix (UROVET Company) according to *NRC (1993)* and this vitamin contains the following: Vitamin B_1 5000 mg/kg, Vitamin $B_2$10.000 mg/kg, Vitamin B_6 5000 mg/kg, Vitamin B_{12}50 mg/kg, Folic acid 1000 mg/kg, Nicotinic acid50.000 mg/kg, Choline 50.000 mg/kg, Pantothenic acid25000 mg/kg, Vitamin $K_3$10.000 mg/kg, Vitamin A**750.000 IU/g, Vitamin D_3 **150.000 IU/g.

Table 2. Calculated chemical analysis (%) of the basal diet.

Chemical analysis	%
Moisture	14.16
Crude protein	34.15
Ether extract	4.18
Crude fiber	4.10
Nitrogen free extract	38.12
Ash	5.29
ME Kcal/kg diet*	3361.724
PE ratio**	101.58

*ME was calculated using a value of 4.5 Kcal/g protein, 8.51 Kcal/g fat and 3.48 Kcal/g carbohydrate according to ***Jauncy and Ross (1982)***.
**Protein/Energy ratio was calculated as mg of protein/Kcal ME according to Jauncy and Ross (1982).

2.3. Experimental design (Table 3)

At the beginning of the experiment, 540 apparently healthy and uniform fish were selected, batch weighed and randomly assigned at 60 fish/group after 1-day starvation. The experiment lasted 84 days; triplicate divisions were randomly assigned to each experimental diet. All dietary treatments were tested in triplicate groups where each aquarium was considered as an experimental unit. During the experiment, all fish were provided by their respective diets at a level of 3% of body weight 6 days a week. The daily ration was divided into three equal amounts and offered three times a day (09:00, 12:00 and15:00 hours). A random sample of 10 fish from each treatment was weighed biweekly and the amount of daily diet was accordingly adjusted.

Table 3. The applied experimental design.

Group	*Diet*	*Supplementation(g/kg diet)*	
		*Probiotics (Tonalast)**	*Prebiotics (AG-FLO)***
1(control)	Basal diet	0	0
2	Basal diet	0.5	0
3	Basal diet	1	0
4	Basal diet	0	0.5
5	Basal diet	0.5	0.5
6	Basal diet	1	0.5
7	Basal diet	0	1
8	Basal diet	0.5	1
9	Basal diet	1	1

***(Tonalast)** The yeast super concentration *(Saccharomyces cervisae)*at concentration of 8 X 10^9 CFU/gram supplied by China Way Corpration, Taiwan, were used.
****(AG-FLO)** Unique combinationofPurified cell wall extraction (MOS) from Sc with aluminoslicate (toxin binding components)supplied by Agresearch INC.

2.4. Evaluation of growth performance

In order to assess the growth performance, sample of tilapia fingerlings were progressively taken in each aquarium. A sensitive electronic balance was used to measure weights to the nearest 0.1 g. weight sample was taken randomly from each aquarium by using a scoop net. The fingerlings were weighed and returned into their respective fingerling aquarium.

The following growth variables: Mean body weight gain (BWG), specific growth rate (SGR), protein efficiency ratio (PER) and food conversion ratio (FCR) and condition factor (CF) were calculated from the biweekly weight data as described by Jauncy and Ross (1982).Body Weight Gain percentage (% BWG) was also calculated as described by Ghosh *et al.* (2003).

2.5. Chemical analysis of diets and fish

Representative samples were taken from the used feed and fish at the start in different experiments Three-whole-fish body from each treatment at the end of the study were taken for proximate chemical analysis to determine the moisture and ash content (AOAC, 1992), crude protein using Kjeldahl method according to Randhir and Pradhan, (1981) and ether extract was determined according to Bligh and Dyer, (1959) technique as modified by Hanson and Olly (1963).

2.6. Blood analysis and immunological parameters

At the end of the experiment, fish were fasted for 24 h prior to blood sampling; blood was collected with a hypodermic syringe from the caudal vein from six fish of each treatment group. The withdrawn blood samples were divided in two sets of Eppendorf tubes. The first set with anticoagulant ((0.1 ml of 4% sodium citrate solution/1 ml blood) used for estimation of

differential leukocytic count (Lucky, 1977), phagocytic activity and phagocytic index (Kawahara et al., 1991),hematocrit value (HT) was also calculated. Total red blood corpuscles (RBC_S) and total leukocytic counts (WBC_S) were determined by haemocytometer according to Maxine and Benjamine, (1985),Hemoglobin content (Hb) was determined by Sahli's method (Lucky, 1977)

The second set was left to clot at 4 °C and centrifuged at 3000 rpm for 15 minutes at room temperature. The collected serum was used for determination of total protein (Doumaset al., 1994), albumin (Reinhold, 1988) using commercial kits produced by Pasteur Lab. Globulin was calculated by subtracting the albumin value from the total protein value of the same sample (Coles, 1998), Albumin/Globulin ratio (A/G) was calculated.

2.7. Challenge experiment

Apparently healthy *O. niloticus* (n=20) from each of treatment group were randomly collected and reared in glass aquaria. *Aeromonas hydrophila* pure strain (locally isolated and identified), kindly supplied by the Poultry and Fish Diseases Department, Faculty of Veterinary Medicine, Alexandria University, was used forbacterin preparation according to Sakai *et al*. (1984). Bacterin injected according to Badran (1990).The treatment groups received two injections of bacterin (formalin inactivated and adjuvant bacterial suspension). Injection was carried out intraperitoneal IP (0.2ml $fish^{-1}$), with an interval two weeks in between the first dose and the booster one. Thereafter (i.e. two weeks after receiving the booster dose); the previously inoculated fish were then artificially challenged with IP injection (0.2 ml of microbial suspension of pathogenic *A. hydrophila* containing 10^8 bacteria/ml). Clinical signs and mortality were recorded for one week. Specificity of death was determined by reisolation of injected bacteria from freshly dead fish during the period of experiment. The relative level of protection (RLP) among the challenged fish was determined (Ruangroupan *et al*., 1986) using the following equation:

RLP % =1 – (% of mortality in treated groups/% of mortality in control group) x100

2.8. Preparation of stained antigen and immune response to *A. hydrophila*

Stained antigen of *A. hydrophila* was prepared (Collins *et al*., 1976). One week post each bacterin and pathogenic *A. hydrophila* injection, two fish were taken from each treatment for blood collection group and blood was collected from the caudal vessel. Serum was then separated in aseptic condition and stored in refrigerator at -20°C (Lucky, 1977) for further assay.Detection of immune response to *A. hydrophila* was evaluated by microagglutination (MA) test where the agglutination titers were expressed as Log_2 of the highest serum dilution still giving a clear agglutination (Badran, 1990).

2.9. Statistical analysis

Statistical analysis was performed using the one way and two ways analysis of variance (ANOVA) to fulfill the requirement of the statistical model

$$X_{ijk}= \mu + T_i + R_j + e_{ijk}$$

X_{ijk}= observed value
μ = population mean
T_i = Effect of treatment I,
R_j = Effect of replicate j
e_{ijk} = random error
Statistical analysis System SAS (SAS Institute Cary, North Carolina, USA, 2002).

3. Results

Results presented in Table 4 represent the effect of *Saccharomyces cerevisiae* as a probiotic and MOS as a prebiotic on the growth of *O. niloticus*fingerlings. After 12 weeks, body weight gain, body weight gain percentages, specific growth rates, feed conversion ratio and protein efficiency ratio were significantly higher ($P < 0.05$) in fish had T_9 (basal diet supplemented with probiotic and prebiotic 1.0/1.0 g/kgdiet). There was insignificant effect ($P > 0.05$) forthe probiotic and/or prebiotic supplemented diet on final body weight, final body length and condition factor (CF).

Proximate body composition (on dry matter, DM basis). revealed a clear increase in DM content of all fish fed the experimental diets when compared with the control group. The crude protein content of fish of T_9 (Sc and MOS-supplemented group: 1/1 g/kg diet) was the highest when compared with all the supplemented Sc and/or MOS-supplemented groups and the control one, followed by T_5 (Sc and MOS-supplemented group: 0.5/0.5 g/kgdiet) at the end of the experiment Table 5.

WBCs and RBCs values for all Sc and /or MOS-supplemented groups were lower than those of control group; however, these decreases were insignificant ($P > 0.05$) (Table 6). On the other hand; the hemoglobin (Hb) and hematocrit (Ht) values were significantly increased ($p < 0.05$) in Sc and MOS -supplemented groups (T2, T7: 0.5/0.0, 0.0/1.0 g/kgdiet respectively) vs. the control. Meanwhile; these values show insignificant differences ($p > 0.05$) in the other Sc and MOS -supplemented treatments.

Serum total protein, albumin, globulin and A/G ratio significantly increased ($p < 0.05$) in (Sc and MOS-supplemented groups 5, 6, 8 and 9

(0.5/0.5, 1/0.5, 0.5/1 and 1/1 g/kgdiet respectively) when compared with control group. In the meantime, there was insignificant difference ($p > 0.05$) in serum globulin between all (Sc and/or MOS-supplemented groups when compared with that of the control group (Table 6).

On regard to the immune response of *O. niloticus* fingerlings among Sc and /or MOS-supplemented groups and the control one, the obtained results revealed that; the phagocytic activity (PA) was significantly increased ($p < 0.05$) in T2 and T7 vs. the control following 12 weeks- experiment, whereas they (Sc and /or MOS-supplemented groups) were insignificantly differed ($p > 0.05$) . Similarly, values of the differential leukocytic count were insignificantly differed ($p > 0.05$) except for the lymphocyte which was significantly higher ($p < 0.05$) in T9 and T5 (Sc and / MOS-supplemented groups: 1.0/1.0 and 0.5/0.5 g /kgdiet respectively) and the monocytes which were significantly higher ($p < 0.05$) in T6 (Sc and MOS-supplemented group, 1.0/0.5 g/kgdiet) (Table 7).

Concerning the challenge experiment; Antibody titers (log_2) of fishes in different treatment groups are presented in Table (8). Higher Antibody titers and lower mortality percentage and higher relative level of protection (RLP%) against *A. hydrophila* post challenge infection were observed in fish of T_3, T_6, and T_9 (Sc and/or MOS-supplemented groups; 1/0, 1/0.5 and 1/1 g/kg diet respectively).

Table 4. Growth performance of Nile tilapia fingerlings after feeding *S. cerevisiae* and/or MOS supplemented diet for 12 weeks(Mean ± SD).

	Treatments (*S. cerevisiae* and/or MOS g/kgdiet)								
Item	T_1 (0/0)	T_2 (0.5/0.0)	T_3 (1/0)	T_4 (0/0.5)	T_5 (0.5/0.5)	T_6 (1/0.5)	T_7 (0/1)	T_8 (0.5/1)	T_9 (1/1)
Initial body weight	34.67± 5.89^{a}	34.78± 4.29^{a}	34.50± 6.60^{a}	35.40± 5.58^{a}	35.35± 3.06^{a}	34.90± 3.15^{a}	35.25± 4.82^{a}	35.85± 5.40^{a}	35.38± 5.28^{a}
Final body weight	58.00± 8.17^{a}	61.00± 5.13^{a}	59.40± 7.57^{a}	62.60± 8.21^{a}	60.30± 6.62^{a}	61.90± 6.11^{a}	61.00± 7.88^{a}	63.30± 8.28^{a}	64.30± 8.35^{a}
Body weight gain	23.33± 3.44^{c}	26.22± 1.66^{abc}	24.90± 1.96^{bc}	27.20± 3.56^{ab}	24.95± 3.74^{bc}	27.00± 3.67^{ab}	25.75± 3.42^{abc}	27.45± 4.47^{ab}	28.92± 3.75^{a}
Body Weight Gain percentage[1]	56.00± 6.47^{b}	61.35± 6.79^{ab}	60.45± 9.63^{ab}	62.80± 8.51^{ab}	57.09± 3.64^{b}	61.87± 5.46^{ab}	59.04± 4.05^{ab}	62.82± 9.73^{ab}	65.70± 6.36^{a}
Specific growth rate[2]	0.62± 0.08^{b}	0.67± 0.06^{ab}	0.66± 0.09^{ab}	0.68± 0.07^{ab}	0.63± 0.04^{b}	0.68± 0.06^{ab}	0.65± 0.05^{ab}	0.68± 0.07^{ab}	0.71± 0.05^{a}
FCR[3]	2.06± 0.03^{a}	1.89± 0.17^{abc}	1.91± 0.28^{abc}	1.83± 0.19^{bc}	1.93± 0.12^{ab}	1.80± 0.20^{bc}	1.88± 0.15^{abc}	1.83± 0.27^{bc}	1.70± 0.18^{c}
PER[4]	2.03± 0.28^{b}	2.20± 0.20^{b}	2.20± 0.32^{b}	2.27± 0.25^{b}	2.14± 0.14^{b}	2.31± 0.23^{b}	2.20± 0.16^{b}	2.29± 0.34^{b}	2.66± 0.56^{a}
Initial body length	12.60± 0.91^{a}	12.69± 0.33^{a}	12.55± 0.77^{a}	12.40± 0.73^{a}	12.42± 0.57^{a}	12.34± 0.62^{a}	12.31± 0.75^{a}	12.40± 0.76^{a}	12.40± 0.74^{a}
Final body length	13.49± 1.33^{a}	13.72± 0.73^{a}	13.80± 0.64^{a}	1400± 0.91^{a}	13.71± 0.67^{a}	13.57± 0.55^{a}	13.66± 1.00^{a}	13.71± 0.81^{a}	14.12± 1.05^{a}
Initial CF[5]	1.73± 0.17^{a}	1.69± 0.12^{a}	1.73± 0.18^{a}	1.81± 0.17^{a}	1.87± 0.37^{a}	1.88± 0.36^{a}	1.92± 0.40^{a}	1.92± 0.55^{a}	1.86± 0.29^{a}
Final CF[6]	2.41± 0.45^{a}	2.37± 0.20^{a}	2.25± 0.10^{a}	2.29± 0.20^{a}	2.37± 0.47^{a}	2.48± 0.18^{a}	2.45± 0.58^{a}	2.51± 0.64^{a}	2.31± 0.41^{a}

Means with different letters at the same row differ significantly at ($p<0.05$).

[1]Body Weight Gain percentage% $BWG = \frac{BWf - BWi}{BWi} \times 100$

[2]Specific growth rate = 100(LN final wt – LN initial wt)/Duration

[3]Feed conservation ratio = dry food consumed in g/Wet wt gain

[4] Protein efficiency ratio = Body gain (g)/Protein consumed (g)

[5] Initial condition factor = Weight $_{initial\ (g)}$/ $(L)^3_{initial\ length}$

[6] Final condition factor = Weight $_{final}$ (g)/ $(L)^3_{final\ length}$(cm)

Table 5. Proximate body composition of Nile tilapia fingerlings after feeding *S. cerevisiae* and/or MOS supplemented diet for 12 weeks.

Treatments(S. cerevisiae and/or MOS g /kg diet)									
Composition % (on dry matter basis)	T_1 (0/0)	T_2 (0.5/0.0)	T_3 (1/0)	T_4 (0/0.5)	T_5 (0.5/0.5)	T_6 (1/0.5)	T_7 (0/1)	T_8 (0.5/1)	T_9 (1/1)
Dry matter	22.85	24.62	25.51	23.94	24.71	23.49	25.58	23.1	24.17
Crude protein	57.4	58.86	57.02	58.31	60.8	57.51	58.8	59.9	63.72
Ether extract	20.85	19.39	18.99	17.5	19.21	18.03	17.28	17.35	13.39
Ash	19.04	18.76	20.63	22.06	16.3	20.3	20.66	19.37	20.24
Carbohydrate	2.71	2.99	3.36	2.13	3.69	4.16	3.26	3.38	2.65

Table 6. Hematological and biochemical changes of Nile tilapia fingerlings after feeding *S. cerevisiae* and/or MOS supplemented diet for 12 weeks (Mean ± SE).

Item	Treatments(*S. cerevisiae* and/or MOS g/kgdiet)								
	T_1 (0/0)	T_2 (0.5/0.0)	T_3 (1/0)	T_4 (0/0.5)	T_5 (0.5/0.5)	T_6 (1/0.5)	T_7 (0/1)	T_8 (0.5/1)	T_9 (1/1)
[1]WBCs (10^3/cm^3)	21.67± 0.56^{a}	22.00± 0.58^{a}	21.67± 0.49^{a}	22.00± 0.58^{a}	22.33± 0.56^{a}	21.50± 0.43^{a}	21.67± 0.33^{a}	21.17± 0.70^{a}	22.00± 0.58^{a}
[2]RBCs (10^6/cm^3)	1.53± 0.04^{a}	1.48± 0.06^{ab}	1.50± 0.06^{ab}	1.33± 0.07^{ab}	1.42± 0.07^{ab}	1.40± 0.06^{ab}	1.30± 0.05^{b}	1.50± 0.10^{ab}	1.45± 0.08^{ab}
[3]Hb (g/100 ml)	7.83± 0.60^{b}	9.67± 0.42^{a}	8.17± 0.40^{ab}	8.67± 0.49^{ab}	8.83± 0.65^{ab}	9.00± 0.58^{ab}	9.67± 0.42^{a}	9.17± 0.48^{ab}	8.50± 0.56^{ab}
[4]Ht %	23.67± 1.17^{b}	27.67± 1.26^{a}	26.67± 1.63^{ab}	26.67± 1.63^{ab}	25.83± 1.30^{ab}	26.50± 0.89^{ab}	28.17± 1.01^{a}	27.00± 1.03^{ab}	26.00± 1.13^{ab}
[5]TP	4.50± 0.10^{cd}	4.50± 0.06^{cd}	4.47± 0.09^{d}	4.90± 0.10^{bc}	5.17± 0.19^{ab}	5.50± 0.06^{a}	4.93± 0.20^{b}	5.17± 0.19^{ab}	5.40± 0.12^{a}
[6]ALB	3.23± 0.09^{e}	3.50± 0.06^{de}	3.43± 0.18^{de}	3.57± 0.20^{cde}	4.13± 0.26^{abc}	4.30± 0.06^{a}	3.67± 0.27^{bcde}	4.13± 0.26^{abc}	4.20± 0.21^{ab}
[7]GLB	1.27± 0.07^{a}	1.00± 0.12^{a}	1.03± 0.12^{a}	1.33± 0.13^{a}	1.03± 0.12^{a}	1.20± 0.00^{a}	1.27± 0.17^{a}	1.03± 0.12^{a}	1.20± 0.26^{a}
[8]A/G ratio	2.54± 0.10^{c}	3.50± 0.07^{ab}	3.33± 0.18^{bc}	2.68± 0.20^{c}	4.01± 0.26^{a}	3.58± 0.06^{ab}	2.89± 0.27^{bc}	4.01± 0.26^{a}	3.50± 0.21^{ab}

Means with different letters at the same row differ significantly at ($p<0.05$).

[1]Total leukocyticcount .
[2]Total red blood corpuscles.
[3]Hemoglobin.
[4]Hematocrite value.
[5]Total protein.
[6]Albumin.
[7]Globulin.
[8]Albumin /globulin ratio.

Table 7. Phagocytic activity, phagocytic index, differential leukocytic count (%) of Nile tilapia fingerlings after feeding *S. cerevisiae* and/or MOS supplemented diet for 12 weeks(Mean ± SE).

Item	*Treatments*(*S. cerevisiae* and/or MOS g/kgdiet)								
	T_1 (0/0)	*T_2 (0.5/0.0)*	*T_3 (1/0)*	*T_4 (0/0.5)*	*T_5 (0.5/0.5)*	*T_6 (1/0.5)*	*T_7 (0/1)*	*T_8 (0.5/1)*	*T_9 (1/1)*
[1]PA	16.67± 0.49bc	19.50± 0.67^{a}	17.00± 0.37^{bc}	19.00± 0.82^{ab}	19.00± 0.58^{ab}	18.50± 0.76^{abc}	19.33± 0.67±^{a}	17.83± 1.05^{abc}	18.17± 0.79^{abc}
[2]PI	1.83± 0.07^{b}	1.90± 0.11^{ab}	2.17± 0.06^{a}	2.13± 0.06^{ab}	2.03± 0.11^{ab}	1.97± 0.08^{ab}	2.00± 0.09^{ab}	2.08± 0.13^{ab}	1.93± 0.12^{ab}
Lymphocyte	45.67± 0.49^{c}	45.50± 0.43^{c}	45.50± 0.56^{c}	46.17± 0.54^{bc}	48.50± 0.76^{ab}	47.50± 0.62^{abc}	45.67± 0.42^{c}	47.50± 1.52^{abc}	49.67± 0.99^{a}
Monocyte	0.83± 0.17^{b}	1.33± 0.21^{ab}	1.17± 0.17^{b}	1.33± 0.21^{ab}	1.50± 0.22^{ab}	2.00± 0.26^{a}	1.00± 0.26^{b}	1.33± 0.21^{ab}	1.00± 0.26^{b}
Basophile	7.50± 0.56^{a}	8.50± 0.43^{a}	8.00± 0.37^{a}	7.67± 0.49^{a}	7.33± 0.49^{a}	8.33± 0.42^{a}	8.67± 0.49^{a}	8.00± 0.37^{a}	8.00± 0.45^{a}
Eosinophile	9.00± 0.58^{a}	8.33± 0.49^{a}	8.83± 0.48^{a}	8.50± 0.43^{a}	8.33± 0.49^{a}	8.00± 0.52^{a}	8.00± 0.58^{a}	8.33± 0.49^{a}	7.83± 0.79^{a}
Neutrophil	36.67± 0.49^{a}	36.33± 0.61^{a}	36.83± 1.05^{a}	34.00± 1.10^{a}	35.33± 1.15^{a}	35.50± 0.81^{a}	36.67± 1.15^{a}	34.83± 1.40^{a}	33.50± 1.78^{a}

Means with different letters at the same row differ significantly at ($p<0.05$).

[1]Phagocytic activity
[2]Phagocytic indix

Table 8. Antibody titers (log_2), mortality percentage and RLP% of Nile tilapia fingerlings after feeding *S. cerevisiae* and/or MOS supplemented diet for 12 weeks.

Item	*Treatments*(*S. cerevisiae* and/or MOS g/kgdiet)								
	T_1 (0/0)	*T_2 (0.5/0.0)*	*T_3 (1/0)*	*T_4 (0/0.5)*	*T_5 (0.5/0.5)*	*T_6 (1/0.5)*	*T_7 (0/1)*	*T_8 (0.5/1)*	*T_9 (1/1)*
1 week After 1st vaccination	4	4	7	4	5	7	5	6	7
1 week After 2nd vaccination	4	4	6	4	5	7	6	5	7
1 week After bacterial infection	4	4	7	4	4	7	6	7	7
Mortality % during 7 days post-challenge	33.3	35.6	8.3	25	25	8.3	16.7	16.7	8.3
Relative level of protection (RLP)%	0.0	-6.9	75.1	24.9	24.9	75.1	49.8	49.8	75.1

4. Discussion

Combination of a pre- and probiotic, termed a symbiotic, is receiving much attention at present in the since this association is thought to improve the survival, activity and efficiency of probiotic bacteria in the GI ecosystem. Symbiotics represent a very new concept for aquaculture. To the best of our knowledge, evaluation of these products has not been conducted to date in aquatic species.

In the present study, the supplementation of commercial live yeast, *S. cerevisiae* as probiotic and MOS as prebiotic (1/1 g/kgdiet) improved, body weight gain (WG) percentages (BWG%), specific growth rates (SPG%), feed conversion ratio (FCR) and protein efficiency ratio (PER). Moreover, higher levels of crude protein content in the musculature of T_9,T_5 fish were recorded.

It can also be noticed that the inclusion of probiotic or prebiotic alone in the diet did not produced a significant improvements in the growth and feed utilization of Nile tilapia fingerlings when compared with the control treatment. Variable results have been obtained with regard to the addition of pre and/or probiotics with respect to increasing growth and feed utilization of tilapia. Abd El-Rhman *et al.* (2009), Ali *et al.* (2010), Ghazala Ali *et al.* (2010), Lara-Flores *et al.* (2010), Abdel-Tawab *et al.* (2008), Marzouk *et al.* (2008), Shoemaker *et al.* (2006), Pirarat *et al. (2008)* and Wang *et al.* (2008) reported that the growth response of Nile tilapia (*Oreochromis niloticus*) was improved following feeding probiotic. On the contrary, He *et al. (2010)*, Abdelhamid *et al*. (2002) reported that the use of probiotic diet supplement did not improve the growth of Nile tilapia.

Concerning prebiotic the results of using different prebiotics supplemented diet is also in -conclusive.It has been reported that the inclusion of inulin (Ibrahem *et al.,* 2010) and MOS (Samrongpan *et al.,* 2008; Schwarz *et al.,* 2010) in the diet of Nile tilapia fingerlings has improved the growth and feed utilization of tested fish. Similar findings were obtained by Hanley *et al.* (1995) who also demonstrated that hybrid red tilapia juveniles, fed 0.6% prebiotic (Aqua-Mos™, Alltech Inc., KY, USA) in their hatchery diets had a 22.5% improved survival with a 27.2% increase in weight gain. On the contrary, Richard *et al.* (2009) concluded that inclusion of prebiotic (oligosaccharide) in tilapia diets at recommended rates from various commercial sources did not show an overall positive effect on the growth performance of juvenile tilapia.

Therefore, the results strongly suggested that combination of both pro- and prebiotics (*S. cerevisiae* and MOS; 1/1 g/kgdiet) as diet supplements has synergistic effect in the growth performance and feed utilization of Nile tilapia. Many prebiotics (FOS, MOS) have been investigated for nutritional manipulation of the GI ecosystem of humans and animals, because they facilitate and support the symbiotic relationship between host and its microbiota (Ferket, 2004; Newman, 2004; Venter, 2007). Moreover, they provide a nutrient source for beneficial bacteria and may promote the maintenance of bifidobacteria and certain Lactobacillus (LAB) in the gut of humans and animals (Mussatto and Mancilha, 2007; Moura *et al*., 2007; Denev, 2008). Consequently probiotics improve fish growth and feed utilization as reported by Kesarcodi-Watson *et al.* (2008), Waché *et al.* (2006) and Ali *et al.* (2010) who found that the addition of probiotic (live yeast) improved diet and protein digestibility, which may explain the better growth and feed efficiency seen with probiotic supplements.

Biochemical analyses often provide vital information for health assessment and management of cultured fish (Pincus, 1996; Cnaani *et al*., 2004; Řehulka *et al*., 2004). In the present study, fish of all treated groups showed improvement in Hb and Ht values in compare to the control treatment. However, only the fish fed diets containing probiotic *(S. cerevisiae*) or prebiotic MOS (T_2 and T_9; 0.5/0, 0/1 g/kgdiet respectively) exhibited significantly higher, Hb, and Ht values. Similarly, Abdel-Tawab *et al.* (2008) reported that feeding yeast supplemented diet (0.5-1.0 g/kgdiet) to Nile tilapia fish resulted in lower Hb and Ht values whereas, fish fed diets containing higher levels (1.0–5.0 g/kgdiet) exhibited higher RBCs, Hb, and Ht values. Moreover, the use of inulin as a prebiotic diet supplement for Nile tilapia fingerlings did not result in remarkable differences in the hematocrit (HT) values and it was a common feature in all tested groups as observed by Ibrahem *et al.* (2010).

The measurement of albumin, globulin, and total protein in serum or plasma is of considerable diagnostic value in fish, as it relates to general nutritional status as well as the integrity of the vascular system and liver function. On this context, the results of this study revealed that the use of both probiotic *(S. cerevisiae*) or prebiotic (MOS) in an increasing manner (T_5, T_6, T_8, T_9) resulted in a significant increase in the serum total protein, albumin, and A/G ratio of tilapia fish which in turn suggest for synergistic effect between probiotic *(S. cerevisiae*) or prebiotic (MOS) when included in the diet of Nile tilapia fingerlings. Whereas the use of probiotic or prebiotic alone did not improve these parameters. These results are in agreement with the results obtained by Mohamed (2007), Yan-Bo *et al*. (2008) and Eid and Mohamed (2010) who concluded that, there was no remarkable difference in the total serum protein, albumin content, globulin contents and A/G ratio between the probiotic supplemented tilapia and the control one. On the contrary, yeast supplementation included in the diet of Nile tilapia increased glucose, lipid, protein, albumin, and globulin values up to 1.0 g kgdiet after which those parameters decreased Abdel-Tawab *et al*. (2008).

Concerning the study results of the non-specific immune response of *O. niloticus* fish groups received

diets supplemented with probiotic *(S. cerevisiae*) and/or prebiotic (MOS), Still the synergistic effect between probiotic and prebiotic is highly suggested as it was clear from the high non-specific immunity developed as manifested by increased number of lymphocytes and monocytes in the differential leukocytic count (T_9, T_5) and (T_6) respectively, as well as increase in the percent of phagocytosis in T_5. These results are in agreement with the results obtained by Jessus *et al*. (2002), Marzouk *et al*. (2008) and Sakai *et al*. (1995) who demonstrated that the use of probiotic supplemented diets enhanced the resistance of fish, by increasing the phagocytic activity of leucocytes. These results could be attributed to the different components of *S. cerevisiae*in particularly the cell wall and β-glucan, which activate the phagocytic cells and melanomacrophages in the hemopiotic organs other than increasing the size of hemopiotic organs as concluded by Marzouk *et al*. (2008). Moreover, Sakai *et al*. (2001) reported that the nucleotides from brewers' yeast RNA were capable of enhancing the phagocytic and oxidative activities of kidney phagocytic cells, serum lysozyme in common carp as well as resistance to *A. hydrophila*. On the other hand, Staykov *et al*. (2005) reported that MOS have a positive effect on non-specific immune response in fish however, the exact mechanisms by which prebiotics enhance the non specific immune response in fish have not been completely elaborated, significant evidence has been accumulated to propose that MOS plays a multi-purpose role in immune modulation (Ferket, 2004; Moran, 2004).

Additionally, the present study revealed that higher levels of probiotics alone /or with increasing level of prebiotic has resulted in higher antibody titers, lower mortality % and higher relative level of protection (RLP%) against *A. hydrophila* post challenge infection as exhibited by treated fish of T_3, T_6, and T_9 (Sc and/or MOS-supplemented groups; 1/0, 1/0.5 and 1/1 g/kgdiet respectively). These results are in agreement with the results obtained by Abdel-Tawab *et al*. (2008.) who concluded that, the use of probiotic (yeast; 1-5 g/kgdiet) as a diet supplement for Nile tilapia resulted in lower mortality% after IP injection of *A. hydrophila*. On the contrary, the study clearly indicated that the use of prebiotic (MOS) alone in tilapia diet did not improve the disease resistance of tilapia against *A. hydrophila* infection. Similarly, Ibrahem, *et al*. (2010) and Wang and Wang (1997) evaluated the efficacies of inulin in the protection of tilapia against *A. hydrophila* infection in vivo. They reported insignificant increase in the survival rates and the number of NBT-positive staining cells of tilapia after infection with *A. hydrophila*.

5. Conclusion

Results of the present study indicate that use of probiotic (*S. cerevisiae*) and prebiotic (MOS) combination as a diet supplement has a synergistic effect in enhancing growth performance and feed utilization of Nile tilapia as well as its resistance to *A. hydrophila* infection. However, future experiments should be designed to measure the subtle effects of oligosaccharide in fish diets with more precision and observations of cellular components of immunity to confirm a significant benefit from these compounds. Regardless of mechanisms of action of oligosaccharide, inclusion of these compounds in fish diets is only warranted if disease resistance, stress tolerance, feed efficiency, or other economic incentives can be reliably proven.

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