



The effects of dietary prebiotic on phagocytic activity of innate immunity in *Oreochromis niloticus*

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ABSTRACT

The objectives of this study, effects of prebiotic β polo on innate immunity of *Oreochromis niloticus* (*O. niloticus*) and increase resist against disease. A total number of 180 Nile-tilapia fish (*O. niloticus*), were divided into four equal groups; the first one was served as control fed on basal non-treated diet. The second group fed on diet supplemented with 1.5 ml prebiotic β polo /kg basal fish diet, the third group fed on diet supplemented with 1 ml prebiotic β polo/kg basal fish diet, and the fourth group fed on diet supplemented with 0.5 ml prebiotic β polo/kg basal fish diet for 40 days. The results showed that, significant increase in phagocytic activity test and total leucocyte count (TLC) levels. The percent level of protection among the three treated groups after challenge infection using *Aeromonas sobria* *Aer. sobria* (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 received 1.5 ml prebiotic β polo /kg basal fish diet. The results of the present study support the use of 1.5 ml prebiotic β polo /kg basal fish diet as fish immunostimulants.

Keywords: Prebiotic, Phagocytosis, *Aeromonas sobria*

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1. INTRODUCTION

Fish are considered the cheapest source of animal protein of high nutritive value especially in developing countries including Egypt. It also a source of essential minerals, vitamins, fat, iodine, fluorine and special amounts of cobalt, magnesium, phosphorous, iron and copper (Gram and Huss, 2000 and Krizek et al., 2004). Intensification of fish farming in aquaculture often leads to deterioration of water quality and this condition makes the cultured organism vulnerable to diseases. Aquaculture faces serious problems due to various adverse effects of antibiotics such as accumulation in the tissue, immune suppression and emergence of resistant microorganisms (Nayak, 2010). Thus

stimulation of the innate immune system by dietary supplement is a suitable approach for fortification of an organism against diseases (Trichet, 2010). Among various immunostimulants, the immune modulatory effects of yeast glucan have already been established in several fish species, The beta-glucans are polysaccharides derived from the cell wall of yeast and fungi. Administration of dietary glucan enhanced growth performance of various fish species (Meena et al., 2012). Moreover, dietary glucan also improved the resistance against bacterial pathogens in several cultured fish species (Jeney G. and Anderson DP., 1997, Misra et al., 2006). The objectives of this study, effects of prebiotic β polo on innate

immunity of *O. niloticus* and increase resist against disease.

2. MATERIALS AND METHODS

2.1. Source of tested fish

Apparently healthy Nile tilapia (*Oreochromis niloticus*) of 25-35 g/fish weight and 9.5-12 cm length were collected from the ponds of the Central Laboratory for Aquaculture Research. Total numbers of 180 fish were kept in previously prepared 12 full glass aquaria (70×60×50 cm), and these aquaria were used for holding the steps of feeding experiment throughout the period of the investigation (40 days). The aquaria were supplied with chlorine free water. The average of water 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 for experimental study.

2.2. Experimental diets of tested fish:

The tested fish were divided into four groups, each group, in three replicates, each 15 fish. The first group (T1), fish were fed with control diet (basal non-treated diet). The second group (T2), was treated with 1.5 ml prebiotic β polo /kg standard fish diets). While, the third group (T3), m was treated with 1.0 ml prebiotic β polo /kg standard fish diets. The fourth group (T4), was treated with 0.5 ml prebiotic β polo /kg standard fish diet). The fish was 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 40 days from the beginning of the feeding experiment

2.3. Microbial strains:

Aeromonus sobria for challenge test was kindly supplied by fish Health and management department, Central Laboratory for Aquaculture Research, Abbassa. *Candida albicans* was kindly supplied by fish Health and management department, Central Laboratory for Aquaculture Research. It was used in a concentration of 5×10^6 CFU/ml to determine the phagocytic activity according to the method described by (Wilinksons, 1977).

2.4. Blood and serum sampling:

Blood samples were collected before and after feeding experiment (every 10 days for 40 days) from the caudal blood vessels of fish, by using syringes previously rinsed in EDTA (15 unit/ml) for the evaluation of TLC according to Feldman *et al.* (2000) by Natt and Herrick solution and phagocytic activity according to the method described by (Wilinksons, 1977).

Separation of white blood cells: White blood cells separation was carried out according to the method described by Anderson *et al.*, (1978). Preparation of *C. albicans* for determining the phagocytic activity according to the method described by (Wilinksons, 1977):

Measuring of phagocytic activity according to the method described by (Wilinksons, 1977):

Phagocytic percentage was calculated according to this formula:

$$\text{Phagocytic \%} = \frac{\text{Number of phagocytic cells engulfing No. of labeled } C. \text{ albicans}}{\text{Total number of phagocytic cells}} \times 100$$

$$\text{P.I.} = \frac{\text{Total number of } C. \text{ albicans in 100 phagocytic cells}}{100}$$

2.5. Challenge test:

The treated as well as the control fish groups were challenged by I/P injection of 0.5 ml 10^7 cells of 24 hrs. cultures of virulent *Aeromonas sobria* at the ends of 40 days' post feed experiment. Fish were observed for two weeks post challenge for mortality rate and the relative level of protection (RLP) calculated according to the equation of (Newan and Majnarichsm1982)

$RLP = 1 - [\text{percentage of treated mortality} / \text{percentage of control mortality}] \times 100.$

2.6. Statistical analysis: According to Murray (1975) by one way ANOVA

3. RESULT

3.1. Total leucocytic count

Effect of prebiotic β polo on total leucocytic count (TLC): Total leucocytic count (TLC) showed significant increase in T2 and T3 in comparison with T₁ all the experiment period and showed no significant change in T4 in comparison with T₁ all the experiment period, as shown in (Table 1). The highest value of TLC obtained at T2 ($19.95 \times 10^3 \pm 9.0$) after 40 days in comparison with T₁ and the lowest value of TLC obtained at T4 ($15.43 \times 10^3 \pm 1.0$) after 10 days in comparison with T₁ (Table 1).

3.2. Phagocytic activity (effect of prebiotic β polo on phagocytic activity):

3.2.1. Effect of prebiotic β polo on phagocytic percentage: -

Cell phagocytic percentage or index was determined in *O. niloticus* fish each 10 days for the experiment period after treatment with different concentration prebiotic β polo. Peripheral blood was collected and phagocytic assay was done as described in the fish. White blood cells

phagocytic activity was determined in *O. niloticus* fish post feeding on prebiotic β polo. Results of table 2 showed, after 10 days and 20 days, Cell phagocytic percentage showed significant increase in T2, T3 and T4 in comparison with T₁. But, after 30 days and 40 days showed significant increase in T2 and T3 in comparison with T₁ and no significant change obtained in T4 in comparison with T₁.

The highest value of phagocytic activity obtained at T2 ($95.66\% \pm 3.480$) after 40 days in comparison with T₁ (photo1) and the lowest value of phagocytic activity obtained at T4 ($64.56\% \pm 2.919$) after 10 days in comparison with T₁.

3.2.2. Effect of prebiotic β polo on phagocytic index:-

Phagocytic index was done to determine the cells capacity to engulf more microbes. Results of table 3 showed, the phagocytic index showed significant increase in T2 and T3 and no significant difference in T4 in comparison with T₁ all the experiment period. The highest value of PI obtained at T2 (25.50 ± 1.89) after 40 days in comparison with T₁ (photo1) and the lowest value of PI obtained at T4 (3.33 ± 0.88) after 10 days in comparison with T₁.

3.3. Mortality rate and Relative level of protection in treated *O. niloticus* after challenge with *Aeromonas sobria*

O. niloticus after challenge with *Aeromonas sobria* showed the highest mortality in T₁ than other treatments that received prebiotic β polo supplemented diet and no mortality after challenge with *Aeromonas sobria* obtained relative level of protection against *Aeromonas sobria* in T₂ was higher than in other treatments (and decreased in the other treatments compared to control (T₁) showed in table (4).

Table (1). Effect of prebiotic β polo supplemented diet on Total leucocytic count (TLC) in *O. niloticus* for forty days of feeding experiment.

Treatments	days	20 days	30 days	40 days
T1	15.72 x10 ³ ± 0.0b	15.63x10 ³ ± 8.0b	15.65x10 ³ ±2.0b	14.10x10 ³ ±1.0b
T2	19.22 x10 ³ ± 3.0a	19.65x10 ³ ±2.0a	19.82x10 ³ ±2.0a	19.95x10 ³ ±9.0a
T3	17.25 x10 ³ ± 8.0a	17.82x10 ³ ± 3.0a	17.93x10 ³ ±1.0a	18.05x10 ³ ±2.0a
T4	15.43 x10 ³ ± 1.0b	16.50x10 ³ ± 6.0b	15.16x10 ³ ± 1b	14.50x10 ³ ±8.0b

Means carrying different superscripts are at ($p \leq 0.05$). The values were given as means \pm 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 (2): Phagocytic percentage in *O. niloticus* phagocytic cells treated with three different concentrations of prebiotic β polo for forty days.

Treatment	10 days	20 days	30 days	40 days
T1	31.66%±0.81b	41.00%±0.57b	53.00%±1.52b	60.66%±0.33b
T2	89.66%±0.47a	91.66%±2.33a	90.33%±0.892a	95.66%±3.48a
T3	87.90%±0.09a	90.76%±1.85a	82.00%±0.736a	82.33%±0.69a
T4	69.00%±0.92a	75.60%±0.43a	64.56%±2.919b	71.00%±0.50b

Means carrying different superscripts are at ($p \leq 0.05$). The values were given as means (\pm 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): Phagocytic index in *O. niloticus* phagocytic cell treated with three different concentrations of prebiotic β polo for forty days

Treatments	10 days	20 days	30 days	40 days
T1	7.36±0.409b	7.03±0.731b	5.90±0.58b	5.33±0.88b
T2	21.43±0.72a	16.86±1.57a	21.03±2.67a	25.50±1.89a
T3	11.83±1.013a	10.76±1.29a	16.88±1.05a	13.11±0.67a
T4	5.11±0.485b	3.66±1.45b	3.33±0.88b	4.00±1.52b

Means carrying different superscripts are at ($p \leq 0.05$). The values were given as means (\pm 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 (4): Mortality rate and Relative level of protection in treated *O. niloticus* after challenge with *Aeromonas sobria*.

treatments	<i>Aeromonas sobria</i>	
	Mortality%	RLP
T ₁	46.66	0
T ₂	0	100
T ₃	6.66	85
T ₄	10	78

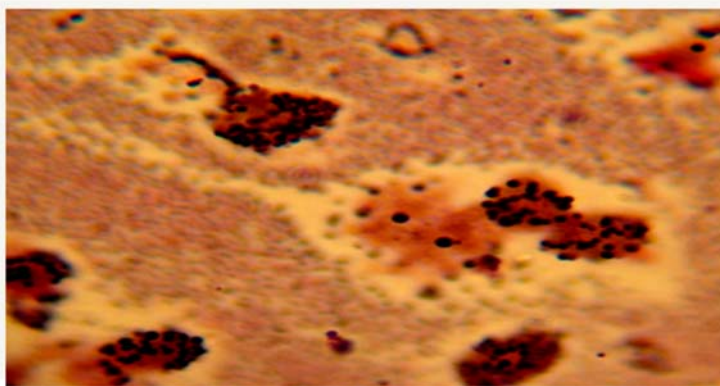


Photo 1: Yeast cells engulfment by phagocytic cells isolated from *O. niloticus* blood, feeding by prebiotic β polo supplemented diet for 40 days of feeding experiment.

4. DISCUSSION

Administration of dietary glucan is a beneficial tool for stimulation of immune system of fish and fortification of immune response (Soltanian *et al.*, 2009). Concerning the non-specific immune stimulation in *O. niloticus* fish groups received diets supplemented with prebiotic β polo. These could be attributed to the components of prebiotic β polo (β -gluca), b-1,3/1,6-Glucans bind specifically to a “receptor molecule” on the surface of phagocytes (Engstadand *et al.*, 1994). b-1,3/1,6-glucans have the same basic biological effect, When the receptor is engaged by b-1,3/1,6-glucan, the cells become more active in engulfing, killing and digesting bacteria and at the same time they secrete signal molecules (*cytokines*) which stimulate the formation of new white blood cells. And also, have the specific immune mechanisms in addition to non-specific defense, the activated phagocytes produce cytokines which also, activate antibody-producing white blood cells (B- and T-cells). Therefore, b-1,3/1,6-glucan enhances also the efficacy of vaccines. Due to the very basic mode of action of b-1,3/1,6-glucans, products in this category affect a number of different biological processes, including not only disease resistance, but also growth (Raa J., 2000). our study agree with Jessus *et al.* (2002) who reported that phagocytic ratio and

phagocytic index rose to their highest level on 42 hrs. after feeding with diet containing 250 mg of Beta-glucan/kg diet. It was clear that high non-specific immunity was developed as manifested by increased number of leucocytic count as well as increase in the percentage of phagocytosis and phagocytic index. The results indicated that TLC, the percentage of Phagocytosis and Phagocytic index in *O. niloticus* in (T2) was the best result, followed by *O. niloticus* in (T3) in comparison to *O. niloticus* in (T1).The present result agree with Misra *et al.* (2006),Yancui Zhao *et al.* (2011) and Sirimanapong W *et al.*(2015) who PI and PC% increase significantly by Glucan.And Selvaraj *et al.* (2006) ,Jamal K. A. *et al.*(2014),Agouz, H. M. and Anwer, W. (2011). And agree Rudabeh Rufchaie and Seyed Hossein Hoseinifar(2014) and (Cuesta *et al.*, 2004)who on the same line of the present study where stated glucan increase significant the TLC .The result of (table 4) mortality rate and relative level of protection of *o.niloticus* after i/p with pathogenic *Aeromonas sobria* agree with Ai, Q., K. *et al.* (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 immunostimulants to enhance the defense potential of fish and shellfish

against bacterial and viral infection and agree with (Abdel-Tawab et al., 2008 and Misra et al., 2006).

5. CONCLUSION

Prebiotic β -polo at 1.5 and 1 ml/kg fish diets have enhance phagocyte activity of innate immunity of (*O. niloticus*) in form of increasing phagocytic capacity, TLC and also increased resistance against *Aeromonas sobria*.

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