رقم البحث (9)

# EFFECTS OF DIETARY INTAKE OF *SACCHAROMYCES CEREVISIAE* ON NONSPECIFIC IMMUNE RESPONSE AND DISEASE RESISTANCE OF NILE TILAPIA (*Oreochromis niloticus*)

***BY***

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## ABSTRACT

Yeast has been known for its effect on health and disease resistance. S. cerevisiae was investigated to detect changes in the activity of innate non-specific immune response and disease resistance of Nile Tilapia Oreochromis niloticus. In this experiment, fish fed on diets supplemented with β-1.3 glucan at a dose of 3.1 g / kg diet and active dried S. cerevisiae whole yeast culture at a dose of 1g/kg diet and control diet for 2 weeks. Total leukocyte count (TLC), Differential leukocyte count (DLC), phagocytosis have been measured as indicators for cellular innate immune response. Lysozyme and protein electrophoresis have been measured as indicators for humoral innate immune response, in addition to immunocompetence assay against Aeromonas hydrophila. The obtained results indicated that, addition of β.1.3 glucan significantly enhances both cellular and humoral immune responses and increases resistance against A. hydrophila infection. While addition of S. cerevisiae as active dried yeast culture enhances mainly cellular immune response and increases resistance to A. hydrophila infection.

Key words : Immunostimulants; Innate immune response; *Oreochromis niloticus*.

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## INTRODUCTION

Immunotherapy is an approach that has been actively investigated in recent years as a method for disease prevention. It is based on nonspecific stimulation of the immune system and it does not involves recognition of a specific antigen but causes an overall immune responses that hasten recognition of foreign proteins **(Secombes, 1994 and Sordillo et al., 1997).** So, the use of immunostimulants for prevention of diseases in fish is considered an attractive and promising area **(Sakai, 1999).**

Fish defense system is similar to that in mammals. For cellular defense, teleost have macrophages, neutrophils, natural killer cells, T and B-lymphocytes **(Iwama and Nakanishi, 1996).** For humoral defense they have complement, lysozyme, hemolysin, transferrin, C reactive protein, interferon and interleukins **(Secombes et al., 1996).**

For a long time, yeast has been known for its effect on health and disease resistance. Yeast cell walls are constructed almost entirely of two classes of polysaccharides; mannoprotein and glucans **(Cabib and Roberts, 1982).** Researches on yeast proved that it was capable of adherence to the gut when supplied with feed and led to enhanced amylase secretion **(Irianto and Austin, 2002).** However, glucan acts mainly by activating the phagocytic cells resulting in increase in production of enzymes that destroy pathogens and chemical messengers (interferon, interleukins and complement proteins) that stimulates immune system **(Raa et al., 1992 and Matsuo and Miyazanoo, 1993).**

The aim of the present work is to study the effect of dietary intake of ***Saccharomyces cerevisiae*** either in the form of β.1.3 glucan (EcoActiva) or active dried whole yeast culture (Biobuds 2X) on innate immune system and resistance to diseases of Nile Tilapia *(Oreochromis niloticus)*.

## MATERIALS AND METHODS

### 1. Fish

A total number of 300 apparently healthy *Oreochromis niloticus* obtained from a private fish farm in EL Dakahlia Governorate with average body weight of 28.12(±5) g transported alive to the laboratory of Department of Fish Diseases and Management, Faculty of Veterinary Medicine, Mansoura University. They were kept for 2 weeks under observation for acclimatization in glass aquaria (40 × 60 × 100 cm). The water of the aquaria was removed daily, and its temperature was maintained at 25 ± 1 °C.

## 2. Immunostimulants

Biobuds 2X®

Biobuds 2X® is an active dried yeast culture of *Saccharomyces cervisiae* along with roughage products carried on calcium carbonate and all ingredients mixed with soybean oil, provided by TOP Vet for import & Export and produced by BROOKSIDE, AGRA L.C., U.S.A. One gram of the product contains 200 million CFU of live yeast cells. It is added to the diet in a dose of one gram $kg^{-1}$ of diet according to the manufacture instructions.

EcoActiva™

EcoActiva™ is a commercial β-1.3 glucan powder preparation. It was kindly provided by Bio-Resources Division, Carlton and United Breweries, Abbotsford Victoria, 3067, Australia. It is manufactured from *Saccharomyces cerevisiae* using a proprietary milling process resulting in a mixture of β 1.3 linked-glucan and mannan with an average particle size of less than 1 μm. The dose was calculated to be 3.1 g of powder $kg^{-1}$ of diet then mixed with the basal diet and pellets were made. The pellets were prepared biweekly, air dried at room temperature and stored in a refrigerator (4 °C) for daily use. It was added to the diet in a dose of according to the manufacture company.

## 3. Experimental design

Fish were divided into 3 groups (100 fish/group). First group served as a control group fed on basal diet, second group were fed on Biobuds® supplemented diet for 2 weeks then fed on basal diet till the end of the 4th week. Third group were fed on EcoActiva® supplemented diet for 2 weeks then fed on basal diet till the end of the 4th week. Each group subdivided into 4 subgroups (25 fish/ subgroup).

Fish were fed on basal diet of 3000 kcal/kg digestible energy and 30% protein either control or immunostimulant supplemented diets at a feeding rate of 30 g diet/kg biomass/day, divided into two feeding times for 2 weeks then fed on immunostimulant free diet till the end of experimental period. The required diets were prepared biweekly and stored in a refrigerator for daily use.

## 4. Determination of nonspecific innate immune response

A total number of 20 blood samples were collected from the caudal vein of 20 fish in each group (5 samples from each replicate) on days 1st, 14th, 21st and 28th of the experimental

period according to **Rowley, (1990).** Each sample divided into 2 haves (one after adding anticoagulant for examination of total leukocyte count and differential leukocyte count using Giemsa stain according to **Schaperclaus, (1992)**. Phagocytosis assay was determined according to **Kawahara et al., (1991)** using *Candida albicans* culture. While the other half allowed to clot at 4 °C, centrifuged at 1500 xg for 10 minutes. The collected serum was frozen at -80 °C until used for lysozyme assay according to **Ellis, (1990)** using 50 µg/ml *Micrococcus lysodictecus* and SDS- *PAGE* serum protein electrophoresis according to **Laemmli, (1970).**

## 5. Immunocompetance test (disease resistance)

On the 28$^{th}$ day of the experimental period, 20 fish from each groups (5 fish/replicate) were injected I/P with 0.1 ml/ 24 hrs broth culture of *A. hydrophila* strain containing $3 \times 10^7$ viable cells/ml according to **(Zaki , 1992),** moralities were recorded daily for 7 days. The relative level of protection (RLP) among the challenged fish was determined according to **Ruangroupan et al., (1986).**

## 6. Statistical analysis

Data were statistically analyzed using one- way or two- way analysis of variance (ANOVA) **(SAS, 1996).** Duncan multiple range test was used to test the significance among the means **(Snedecor and Cochrang, 1989).** Differences were considered significant at $P < 0.05$.

# RESULTS

## 1. Assessment of cellular innate immune response

### 1.1. Total leukocytes count (TLC)

There was a high significant increase ($p < 0.01$) in TLC reached the highest level at the end of 3rd week of experiment with β-glucan (EcoActiva™) and yeast (Biobuds®) supplemented group compared to control (fig.1).

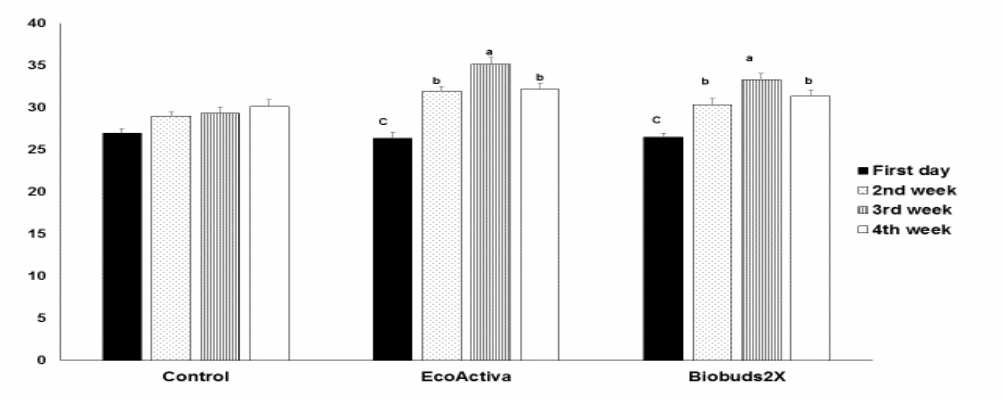
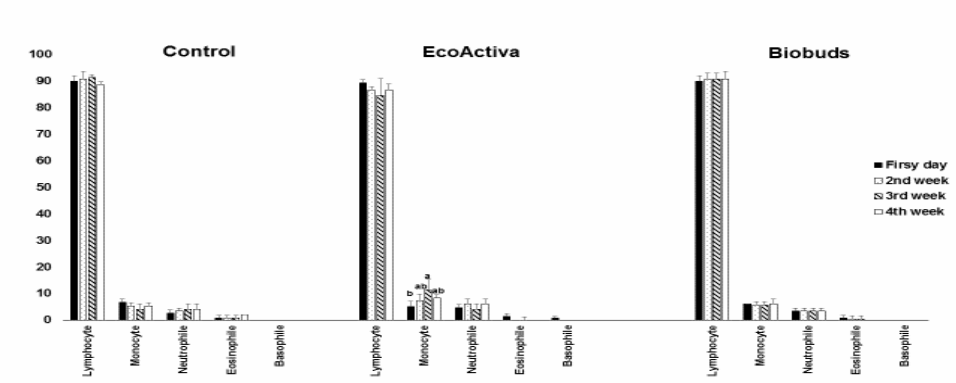



**Fig. 1.** Total leukocytes count (cells x$10^3$ cell/µl) of *O. niloticus* fed on different immunostimulants for 2 weeks.

Columns with the different letter are significantly different at P<0.05.

### 1.2. Differential leukocytes count (DLC)




As shown in fig. 2, monocytes % of *O. niloticus* fed on EcoActiva supplemented diet showed a significant increase peaked at the end of the 3rd week compared to control or Biobuds treated diets. Other cell types were non-significant in all treatments including control throughout the experimental period.

**Fig. 2.** Differential leukocyte count of *O. niloticus* fed on different immunostimulants for 2 weeks.

Columns with the different letter are significantly different at P<0.05.

## 1.3. PHAGOCYTOSIS

Phagocyte activity in all groups was non-significant, while phagocyte index significantly increased during the experimental period peaked after 3rd week for β-glucan supplemented group (EcoActiva) followed by whole yeast supplemented group (Biobuds 2X) compared to control (fig.3).

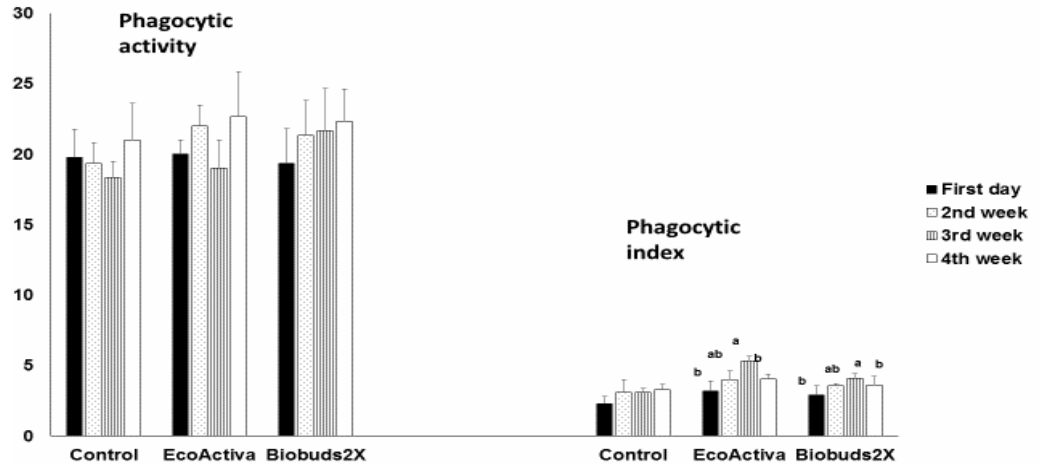



**Fig. 3.** Phagocytosis of *O.niloticus* fed on different immunostimulants for 2 weeks.

Columns with the different letter are significantly different at P<0.05.

### 1.4. Assessment of humoral innate immune response

#### *1.4.1. Lysozyme concentration*

Lysozyme concentration significantly increased with Ecoactiva supplemented group reached its maximum level at the end of 2nd week with compared to other treatments including control (fig. 4).

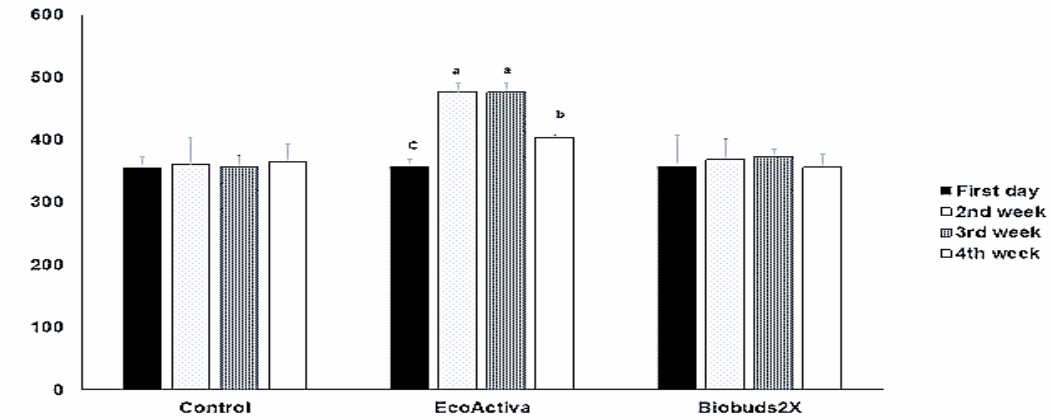



**Fig. 4.** Lysozyme concentration (µg/ml) of *O.niloticus* fed on different immunostimulants for 2 weeks.

Columns with the different letter are significantly different at P<0.05.

### *1.4.2. Protein electrophoresis*

γ-Globulin concentration significantly increased at the end of the 3rd week with EcoActiva (at $p < 0.05$) in fig.5, while non-significant with Biobuds. The total albumin, α and β globulin didn't show a significant difference either in each treatment throughout the experimental period, or between different treatments including control (data not shown).

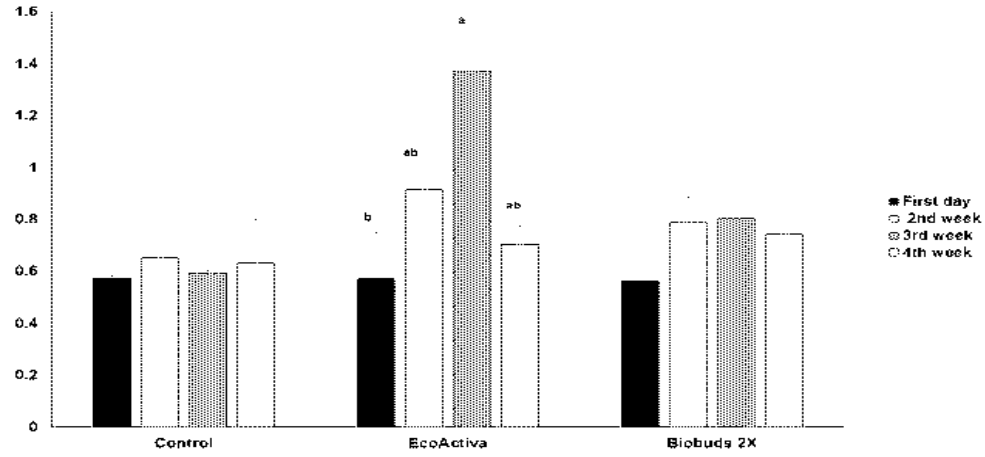



**Fig. 5.** Protein electrophoresis (globulin) of *O. niloticus* fed on different immunostimulants for 2 weeks.

Columns with the different letter are significantly different at P<0.05.

## 5. IMMUNOCOMPETANCE TEST (DISEASE RESISTANCE)

Mortality rates have been decreased with Ecoactiva supplemented group to 10% and high relative protection level (87.5) while was 30% with Biobuds supplemented group with moderate RPL (62.5) compared to 80% mortalities and no protection in control group table (1).

**Table 2.** Disease resistance of *O. niloticus* fed on different immunostimulants for 2 weeks.

| Fish groups | No. of fish | Route of injection | Type of Inoculate | Dose (ml) | Died fish during 7 days after injection. | | | | | | | | Mortality (%) | RLP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | | |
| Control | 20 | I/P | *A. hydrophila* | 0.1 | _ | 4 | 3 | 3 | 2 | 2 | 3 | 1 | 80 | 0 |
| EcoActiva | 20 | I/P | *A. hydrophila* | 0.1 | _ | _ | 1 | _ | 1 | _ | _ | _ | 10 | 87.5 |
| Biobuds 2X | 20 | I/P | *A. hydrophila* | 0.1 | _ | 1 | 2 | _ | 1 | _ | 2 | _ | 30 | 62.5 |

## DISCUSSION

Natural immunostimulants are biocompatible, biodegradable and safe for the environment and human health. Moreover, they possess an additional nutritional value **(Sahoo and Mukheree, 2002).** Yeasts have been used in aquaculture as a probiotic due to their fast growth, low cost and high stability **(Irianto and Austin, 2002).** Results of the presnt study indicated that the dietary intake of ***Saccharomyces cerevisiae*** either as a β-1.3glucan at dose of (3.1 g / kg diet) for 2 weeks or whole yeast in a dose of (1 g / kg diet) significantly enhanced cellular immune response (TLC), monocytes % and phagocytosis of ***O. niloticus***. Similarly, **(Siwicki et al., 1994)** in rainbow trout reported an increase in TLC, P.A and P.I with dietary intake of *β-1.3* glucan for 1 week. In addition, glucan treatment showed enhanced phagocytic cell activities which can be detected by phagocytosis (**Jorgensen et al., 1993).** This may be attributed to the mode of action of glucan on phagocytic cells which activated after engagement of β-glucan to its specific receptors on surface of phagocytic cells. Supporting this hypothesis, **Cook et al., (2001)** reported that, recognition of β.1.3 glucan of EcoActiva and subsequent modulation of macrophage activity most likely occurs through engagement of these specific receptors. For whole yeast immunostimulation, the enhanced cellular activity could be attributed to the presence of glucan receptors on the cell surface of blood monocytes, macrophages and neutrophiles. More over the intake of chitin also increases the head kidney immune response, including phagocytosis **(Esteban et al., 2001).**

For humoral immune response, ***O.niloticus*** fed on β-1.3 glucan supplemented diet showed an increased serum lysozyme concentrations and γ-globulin levels while had a non-significant effect on total protein and total albumin levels. The results obtained by **(Bagni et al., 2000)** in sea bass ***D.labrax*** reported a significant increase in lysozyme concentrations after feeding a diet supplemented with 2% β-1.3 glucan over a 2-weeks period, every 3months. While have non-significant effect on protein content or in albumin/ globulin ratio. In contrast to these findings, feeding with ***S. cerevisae*** resulted in no increase in lysozyme activities **(Verlhac et al., 1998),** which agrees with the results obtained from this study. This different effect of the same immunostimulantion on humoral defense depending of the administration method which probably related to the induction of an inflammatory reaction and its consequent synthesis of acute phase proteins (APPs), because the oral administration does not appear to induce APPs while injection does **(Robertsen, 1999 and Ortuno et al.,**

**2002).** Therefor it is not surprising that the administration of yeast by injection should enhance the humoral defense. However, further experiments in this line are needed.

Concerning protection against diseases, β-1.3 glucan supplemented diet increased protection of ***O. niloticus*** against ***A. hydrophila*** infection, as it caused a maximum reduction in mortality rate to 10% compared to 30 % with whole yeast supplementation and 90% in control (+ve). Many studies similarly recorded increased resistance against ***A. hydrophila*** infection with the dietary intake of β- 1.3 glucan in different fish species as carp **(Siwicki et al., 1994a)** and African cat fish **(Yoshi da et al., 1995)**. This may be attributed to the increase activity of phagocytic cells with subsequent increase in lysozyme activity and bactericidal activity of fish phagocytes / macrophages **(Robertsen et al., 1994).** Furthermore, **(Cook et al., 2001)** demonstrated that β-1.3 glucan of EcoActiva was highly effective in priming macrophages to subsequent stimulation with LPS which is the integral component of cell wall of Gram negative bacteria such as ***Aeromonas, Vibrio, and Yersinia Species*** .

In conclusion, addition of β-1.3 glucan of ***S. cerevisiae*** to the diet of ***O.niloticus*** at a dose of 3.1 g/kg of diet for two weeks significantly enhances both cellular and humoral immune responses and increases resistance to ***A. hydrophila*** infection rather than using whole yeast cell.

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# الملخص العربى

# تأثير استخدام السكارومايسس كاضافات اعلاف على الحالة الصحية ومقاومة الأمراض لأسماك البلطى النيلى

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أجريت هذه الدراسة بهدف دراسة تأثير السكارومايسس المختلفة على الأستجابة المناعية غير المتخصصة ومقاومة العدوى بميكروب الأيروموناس هيدروفيلا لأسماك البلطى النيلى.

فى هذه التجربة تم تغذية أسماك البلطى النيلى (٢٨±٥) جرام وزن متوسط على علائق مختلفة مزودة بالمنشط المناعى الخميره بجرعة ٢٢٥ مللى جرام لكل كيلو جرام عليقة والبيتاجلوكان بجرعة واحد جرام لكل كيلو جرام عليقة . تم تغذية الاسماك لمدة أسبوعين بمعدل ٣٠ جرام من العليقة لكل كيلو جرام من الوزن الحي للسمكة يوميا. وقد وجد أن اضافة البيتاجلوكان او البيوبدز أدت الى زيادة الاستجابة المناعية الخلوية من حيث زيادة في العدد الكلي لكرات الدم البيضاء وعدد خلايا المونوسايت بالاضافة إلى زيادة قدرت الخلايا الاكولة النشطة على التهام فطر الكانديدا البيكانس والتى وصلت لأعلى معدلاتها عند نهاية الاسبوع الثالث بالأضافة الى زيادة الاستجابة المناعية غير الخلوية حيث أدت إلى زيادة تركيز وكفاءة الانزيمات المحللة في المصل والتي وصلت أعلى مستوى لها عند نهاية الاسبوع الثالث ولكنها لم تكن معنويه التاثير باستخدام البيوبدز بجانب ارتفاع معنوي ملحوظ في الجاماجلوبيلين والذي وصل الى اعلى مستوى له عند نهاية الاسبوع الثالث مع انخفاض معدل النفوق الناتج عن العدوى البكتيرية ببكتيريا الايروموناس إلى١٠٪ مقارنة بالمجموعة الضابطة الموجبة والذى وصل الى ٩٠٪ بينما ادت أضافة البيوبدز الى انخفاض معدل النفوق إلى٣٠٪ مقارنة بالمجموعة الضابطة الموجبة .