

Use of Probiotics as Growth Promoter, Anti-Bacterial and Their Effects on the Physiological Parameters and Immune Response of *Oreochromis Niloticus* Lin. Fingerlings

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ABSTRACT

A growing concern for the high consumption of antibiotics in aquaculture has initiated a search for alternative methods of disease control. Improved resistance against infectious diseases can be achieved by the use of probiotics. The objective of the present study was to evaluate the influence of some probiotics on the growth performance, physiological measurements, immune response and economic efficiency of Nile tilapia (*Oreochromis niloticus* Lin.) fingerling diets. The experimental fish were fed 9 diets supplemented with 0.05, 0.10 and 0.15% with Super Biobuds; Bio-yeast and Stop stress gold at 0.5, 1.0 and 1.5 g / kg diet and T0 as a control group fed free probiotic diet for 70 days. The feeding rate was 5% of the total body weight divided at 4 times daily. Then, the experimental fish were divided into two groups; the first was fed on diets supplemented with three different levels of previous probiotics and the second group was served as control that fed free probiotics diet for two weeks to evaluate immune response. Also, heparins blood samples were collected for measuring of blood parameters. The results indicated that fish groups fed on diets supplemented with probiotics revealed significant increase in body weight gain and concentrations of serum protein, globulin and enhancing immune responses (non specific immune response as detected in vitro phagocytes' activity test). So, it could be concluded that dietary probiotics can impart beneficial effects on Nile tilapia growth and that translates into financial benefits for farmer, by decreasing feed cost per unit growth of fish.

Keywords: Nile tilapia, probiotic, physiological measurements and immune response

INTRODUCTION

The demand for animal protein in Egypt is expected to increase progressively with each increase in human population. Nile Tilapia have been called opportunistic omnivorous, that they well consume a variety of feed, living or drying materials, animal or plant (Stickney, 1997). Aquaculture is one of the most important options in animal protein production and requires high quality feeds with high protein content as well as some complementary additives to keep organisms healthy and favor growth. The use of probiotics as farm animal feed supplements dates back to the 1970. One of the best methods to reduce feed cost is through the use of feed additives (Lara - Flores *et al.*, 2003). Their primary effects are to improve feed efficiency and / or daily gain. The use of these additives allows fish farmers to maximum performance through improvement in health, gain, and reproduction and feed efficiency (Marzouk *et al.*, 2008).

Continuous feeding of probiotics for a period of time rather than dosing is recommended as it appears most probiotics will not permanently establish themselves in the gut (Kumprecht, and Zobac, 1995). They were originally incorporated into feed to increase the animal's growth and improve its health by increasing its

resistances to disease. The results obtained in many countries have indicated that some of the bacteria used as probiotics (*Lactobacilli*) are capable of stimulating the immune system (Fuller, 1992). It was clear that experience obtained with terrestrial animals has been used in aquaculture, especially with regard to the use of lactic acid bacteria, there were some articles associated with several modes of probiotics action (Fukami *et al.*, 1997).

The microorganisms used as probiotics, including yeasts, lactic acid bacteria, *Pseudomonas*, have been evaluated in aquatic animals (Balcazar *et al.*, 2007). Among lactic acid bacteria, including some *Enterococcus faecium* strains are non-pathogenic, with an ability to produce lactic acid and bacteriocine (Herranz *et al.*, 2001). Probiotic preparations of *Enterococcus faecium* preparation have received more interest in animal management; whereas they can stimulate immune system and protect animals from gastrointestinal diseases (Taras *et al.*, 2006). Hence, Wang *et al.* (2008) found that the addition of *Enterococcus faecium* in aquaria water could increase the growth performance and improve the immune response of the tilapia. Owing to some problems and limitation in using hormones and antibiotics for animals and the final

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consumers, probiotic bacteria are a good candidate for improving the digestion of nutrients and growth in aquatic organisms (Irianto and Austin, 2002 and Lara-Flores *et al.*, 2003). The effects of some bacteria strains have been studied by (Lara-Flores *et al.*, 2003) they found that all the probiotic - supplemented diets resulted in higher growth than non-treated diets. The nutrients in organisms could be improved by the detoxification of potentially harmful compounds in the diet by hydrolytic enzymes, including amylases and proteases and the production of vitamins such as biotin and vitamin B₁₂ (Irianto and Austin, 2002). Marzouk *et al.*, 2008 reported that, Nile tilapia fed on probiotics had higher resistance on challenge with pathogenic bacteria. Fish diseases are major problem for the fish farming industry and among those bacterial infections are considered to be a major cause of mortality in fish (Gomez-Gil *et al.*, 2000). The motile aeromonads, especially *A. hydrophila*, affects a wide variety of Freshwater fish species and occasionally marine fish (Chu and Lu, 2005). Thus, many measures have been tried to improve Production levels such as the routine use of antibiotics. However, the excessive and inappropriate use of antibiotics has resulted in the presence of resistant strains of bacteria in fish culture (Nomoto, 2005). In addition, there are

environmental problems associated with the antibiotics (Wang and Xu, 2004). Therefore, the need for alternatives is increasing and the contribution of Probiotics may be considerable with increasing demand for environment friendly aquaculture, the use of probiotics in aquaculture is now widely accepted (Vine *et al.*, 2006; Kesarcodi-Watson *et al.*, 2008). Probiotic bacteria have a possible competition with pathogens (Gatesoupe, 1997) or the hypothetical stimulation of the Immune system, as the activation of macrophage (Spanggaard *et al.*, 2001; Wang *et al.*, 2008). Lactic acid bacteria (LAB) are great producers of bacteriocins and organic acids (lactic and acetic acids) which have inhibitory effects *in vitro* on the growth of some pathogens in fish (Planas *et al.*, 2004) and these antimicrobial substances have provided these organisms with a competitive advantage over other microorganisms to be used as probiotics (Salminen *et al.*, 1998). A considerable interest in the use of LAB as probiotics for improving disease resistance, growth of fish and in enhancing fish immune response has been developed (Zhou *et al.*, 2010). So that, the objective of this study to investigate the effects three different levels of three commercial probiotics on the growth performance, hematological and immunological efficiency of Nile tilapia fingerlings.

MATERIALS AND METHODS

Feeding trial was carried out in Central Laboratory for Aquaculture Research, Abbassa, Abou- Hammed, El-Sharkia Governorate, Egypt. Nile tilapia (*O. niloticus* Lin.) fingerlings fed at a rate 5% of the total biomass of fish daily (4 times a day at 9.00, 12.00, 15.00 and 18.00 hr). The amount of diet was adjusted biweekly according to the change in fish weight.

Experimental probiotics

This trial consisted of ten treatments (10 diets) to investigate the effects of three probiotics {Super Biobuds (P1), Bio-yeast (P2) and Stop stress gold (P3)} on the growth performance, physiological measurements and immune response of Nile tilapia (*O. niloticus* Lin.) fingerlings. The probiotics (P1, P2 and P3) were added to Nile tilapia fingerlings diets at three levels 0.5, 1, and 1.5 g/kg diet. The control group received the basal diet free of probiotic supplementation. All experimental diets were formulated to cover all nutrients requirements by Nile tilapia as recommended by National research council (NRC, 1993).

The dietary ingredients and their composition are show in Table (1)

were finely ground, weighed according to their percentage and mixed together then 30% boiled water was added to each diet to be easily pelleted by pressing through 1 mm diameter by pelleting unit. The pellets were dried in a drying oven at 60 °C for 24 hours and stored at – 4 °C until use during the trial to avoid oxidation and rancidity. The dietary ingredients and the experimental diets were analyzed according to standard methods of Association of Official Analytical Chemists (AOAC, 1990) for moisture, protein, total lipids, and ash. Moisture constant weight at 85 °C in drying oven (GCA, model 18 EM, Precision Scientific group, Chicago, Illinois, USA). Nitrogen content was measured using a microkjeldahl apparatus (Labconco, Labconco Corporation Kansas, Missouri, USA) and crude protein was estimated by multiplying nitrogen content by 6.25. lipid content was determined by ether extraction in multi-unit extraction Soxhlet apparatus (Lab. Line Instruments, Inc., Melrose Park, Illinois, USA) for 16 hours and ash was determined by combusting dry samples in a muffle furnace (Thermolyne Corporation, Dubuque, Iowa, USA) at 550 °C for 6 hours.

The chemical analysis of the experimental diets is show in Table (2).

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Table (1): The composition of the experimental diets.

Ingredients	Control	Super Biobuds (P ₁) g/kg			Bio-yeast (P ₂) g/kg			Stop stress gold (P ₃) g/kg		
		0.5	1.0	1.5	0.5	1.0	1.5	0.5	1.0	1.5
Fish meal	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2
Soybean meal	52.5	52.5	52.5	52.5	52.5	52.5	52.5	52.5	52.5	52.5
Yellow corn	19.5	19.5	19.5	19.5	19.5	19.5	19.5	19.5	19.5	19.5
Starch	8.00	7.95	7.9	7.85	7.95	7.9	7.85	7.95	7.9	7.85
Corn oil	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
Fish oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Vit*.& Min.**	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Cellulose	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Probiotic	--	.05	0.1	0.15	0.05	0.1	0.15	0.05	0.1	0.15

*- Vitamin premix (per kg of premix): thiamine, 2.5g; riboflavin, 2.5g; pyridoxine, 2.0g; inositol, 100.0g; biotin, 0.3g; pantothenic acid, 100.0g; folic acid, 0.75g; para-aminobenzoic acid, 2.5g; choline, 200.0g; nicotinic acid, 10.0g; cyanocobalamine, 0.005g; a-tocopherol acetate, 20.1g; retinol palmitate, 100.000 IU; cholecalciferol, 500.000 IU.

**-. Mineral premix (g/kg of premix): CaHPO₄·2H₂O, 727.2; MgCO₃·7H₂O, 127.5; K.Cl, 50.0; NaCl, 60.0; FeC₆H₅O₇·3H₂O, 25.0; ZnCO₃, 5.5; MnCl₂·4H₂O, 2.5; CuCl₂, 0.785; CoCl₃·6H₂O, 0.477; CaIO₃·6H₂O, 0.295; CrCl₃·6H₂O, 0.128; AlCl₃·6H₂O, 0.54; Na₂SeO₃, 0.3 g

Table (2): The chemical composition of the experimental diets

Items	P ₁ g/kg			P ₂ g/kg			P ₃ g/kg			Control
	0.5(T ₁)	1.0(T ₂)	1.5(T ₃)	0.5(T ₄)	1.0(T ₅)	1.5(T ₆)	0.5(T ₇)	1.0(T ₈)	1.5(T ₉)	
DM	92.84	92.34	92.04	92.27	92.00	92.11	92.26	92.43	92.14	92.30
CP	30.14	30.07	29.91	29.97	29.87	30.02	30.11	29.98	30.15	29.94
EE	9.68	9.89	9.66	9.77	9.95	9.78	9.82	9.90	9.76	9.98
Ash	7.40	6.64	6.39	6.68	6.85	6.37	6.75	6.63	6.91	7.24
CF	5.49	5.51	5.39	5.49	5.19	5.67	5.32	5.11	5.47	5.55
NFE*	47.29	47.89	48.65	48.09	48.14	48.16	48.00	48.38	47.71	47.29
GE** (kcal/100g)	455.66	459.71	459.74	458.83	460.17	459.49	459.72	461.3	458.19	457.36
P/E ratio	66.15	65.41	65.06	65.32	64.91	65.33	65.50	64.99	65.80	65.46

* Nitrogen free extract (NFE) = 100 - (protein% + lipid% + ash% + crude fiber)

** Gross energy (GE) was calculated as 5.65, 9.45 and 4.11 K Cal/g for protein, lipid and NFE, respectively (NRC, 1993).

Experimental fish

All male Nile tilapia (*O. niloticus* Lin.) fingerlings were obtained from Abbassa Hatchery Company, Abou Hammad, El-Sharkia Governorate. They seemed healthy and had an average body weight of 5.00 ± 0.218 g/fish and length about 3.5 ± 0.514 cm. A total 450 fish were used during the experiment (45 fish in each treatment) which distributed randomly into 30 glass aquaria. Each treatment including 3 replicates (aquaria) in which 15 fish were stocked in each aquarium.

Growth parameters

Nile tilapia fingerlings were weighed biweekly during the experimental period (70 days). Total weight was determined to the nearest gram according to Annet (1985).

Experimental system

Glass aquaria measuring (80 x 60 x 40 cm) of 120 liters each were used to stock Nile tilapia (*O. niloticus* Lin.) fingerlings, which acclimatized to the lab conditions for 2 weeks. Then, thirty experimental glass aquaria were used for 10 treatments to execute feeding trial. Each aquarium was supplied with an air pump contacted with two air stones for aeration. Tap water has been stored 24 hours in fiberglass tank for dechlorination and filling the aquaria after replacing at

100% of water daily. Water temperature (via a thermometer) was daily measured and the average was between 27.5-28.5°C during the experimental period. Water quality parameters were measured weekly. The pH (using Jenway Ltd., Model 350-pH-meter), ammonia were estimated during the experimental feeding period according to APHA (1995) and dissolved oxygen (using Jenway Ltd., Model 970- dissolved oxygen meter).

Probiotics

Three preparations of commercial probiotics were used as sources of *Lactobacillus acidophilus*, *Lactobacillus casei*, Lactis and *Bifidobacterium bidum* plus bacterial nutrients, yeast (*Saccharomyces cerevisiae*) to test their effects on the growth performance and feed utilization of Nile tilapia (*O. niloticus* Lin.) fingerlings. The composition of these probiotic preparations, as claimed by the manufactures, is as:

Super Biobuds (P1)

1-Super Biobuds composition

Active	dry	yeast	6%
<i>(Saccharomyces cerevisiae)</i>			
Extruded wheat middling		45%	
Soybean oil		0.50%	
Calcium carbonates		48.5%	

Contains 800 million CFU/g live cell yeast.

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2-Bio-yeast (P2)

Active viable microorganisms

<i>Saccharomyces cerevisiae</i>	5 billion cells / g
<i>Lactobacillus acidophilus</i>	5 billion cells / g
<i>Streptococcus faecium</i>	5 billion cells / g
<i>Lactic acid bacteria</i>	2.0%
<i>Aspergillus oryzae</i> fermentation	27.5
Yeast fermentation extract	33.5%

3-Stop stress gold (P3)

Lactobacillus acidophilus,

Lactobacillus caseii,

Lactic and *Bifdo bacterium Bedim* plus bacterial nutrients and yeast.

The P3 contained 1 billion CFU/g Lactic acid beneficial bacteria.

The stability of the product is kept by storage in cool, dry conditions to maintain its probiotics for 24 months.

Physiological measurements

At the end of the feeding trial, fish blood samples were collected with a hypodermic syringe from the caudal vein. The blood was divided in two sets of Eppendorf tubes. One set contained sodium heparinate (20 UL⁻¹), used as an anticoagulant, for

hematology (hemoglobin, hematocrit, and red blood cells counting) while the second set, was left with no anticoagulant in order to clot at 4 °C and centrifuged at 5000 rpm for 10 min. at room temperature. Red blood cells (RBCs) were counted under the light microscope using a Neubaver haemocytometer after blood dilution with phosphate-buffered saline (pH 7.2). Hematocrit values (Ht) were immediately determined after sampling by placing fresh blood in glass capillary tubes and centrifuged for 10 min. in a micro-hematocrit centrifuge (Damon / IEC division, Needham HTS, Mass, USA) then measuring the packed cell volume. Hemoglobin levels (Hb) were determined calorimetrically by measuring the formation of cyanomethemoglobin according to Van Kampen and Zijlstra (1961). Glucose was determined calorimetrically according to Trinder (1969). In serum, total protein was determined calorimetrically according to Henry (1964). Total lipids were determined calorimetrically according to Joseph *et al.* (1972). Urea was measured according to Barham and Trinder (1972). Albumine was determined calorimetrically according to Wotton and Freeman (1982). Globuline was obtained by the subtraction of albumin from total protein. Activities of aspartate

aminotransferase (AST), alanine aminotransferase (ALT) were determined calorimetrically according to Reitman and Frankel (1957) creatinine and by using a spectrophotometer (model 5010, Germany) and commercial kits.

The immune response of fish as affected by treatments

Challenge test

At the end of the feeding trial, six fish in each aquarium (total 180) were divided into two groups. The first group was challenged with pathogenic *Aeromonas hydrophila* (*A. hydrophila*) from natural outbreaks in fish farm kindly provided by fish diseases dept. Central Laboratory for Aquaculture Research (CLAR) 0.1 ml dose of 24-h saline from virulent bacterial pathogen of *A. hydrophila* (5×10^5 cells/ml) was given by interperitoneal (IP) route (Schaperclaus *et al.*, 1992). The second group was IP injected by 0.1 ml of saline solution as a control. All groups were kept under observation for 15 days to record clinical signs and daily mortality rates.

Economical evaluation

The economical efficiency of using three probiotics in Nile tilapia fingerlings diets calculated as:

$$\text{Cost of feed fed} = \text{Price of diet} \frac{\text{LE}}{\text{kg}} \times \text{Feed intake (g)}$$

$$\text{Income from gain (LE)} = \text{Total gain} \times \text{Price of Kg live body weight}$$

The estimation was based on local retail sale market price of the entire dietary ingredient at the time of the study (2007). These prices (in LE/kg) were as follows: herring fish meal, 8.00; soybean meal, 3.70; corn meal, 2.75; wheat bran, 1.30; starch, 6.00; corn oil, 5.50; premix, 8.00; cellulose, 3.50, and price of selling of one Kg live body weight of fish was 8.00 LE in 2007.

Statistical analysis

The data of the present study were analyzed using the SAS Programme (1992) SAS/STAT User's guide Release 6.03 Edition SAS Inst. INC. Cary, NC, USA, considering the control group for comparison. Source and level of probiotics were the main comparison effects either for the feeding or the immunity experiments. Duncan's new multiple range test was conducted to determine the significant differences ($P < 0.05$) between means (Duncan, 1955), accordingly:

The used model for analysis was

$$X_{ij} = \mu + T_i + E_{ij}$$

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Where

μ is the overall mean

T_i is the effect of all treatments including the control.

E_{ij} is the experimental random error.

RESULTS AND DISCUSSTION

All tested water quality criteria were suitable for rearing Nile tilapia *O. niloticus* fingerlings. Since water temperature ranged between 27.5 and 28.5°C, pH values 7 – 7.8, ammonia (NH₃) from 0.03 to 0.04 mg / l. and dissolved oxygen 5 – 6.2 mg/l. the results are similar to those Abdel-Hakim *et al.* (2002b).

Data presented in Table (3) indicated that the highest final body weight was obtained by T₁ group which received diet supplemented with 0.5 g/kg followed by T₅, T₉, T₂, T₆, T₃, T₄, T₇ and T₀ groups, respectively. The lowest values were obtained by T₈, but the differences between T₆, T₇, T₀ and T₈ were not significant (P>0.05). In case of T₁, T₂ and T₃ groups, final body weight was higher in T₁ group (0.5g/kg diet). Thus, final body weight reached 27.1 g/fish (T₁) followed by T₂ (24.2g/fish) and T₃ (22.7g/fish). Nile tilapia fingerlings which fed on the diet supplemented with Bioyeast (P2) at 0.1% /Kg diet also recorded the highest final live body weight being 25.4g /

fish, which was insignificantly higher than those obtained by 1.5g P2 /Kg diet and 0.5g P2 /kg diet. It appears from the present results, adding the commercial probiotics in tilapia diets increased final body weight (except T₈) compared with control group(T₀).In the respect, Abd El-Halim *et al.* (1989) found that the addition of living yeast in diet improved the growth performance of *O. niloticus*. Also Scholz *et al.* (1999) reported that *S. cerevisiae* improved the growth and survival of juvenile *Penaeus vannamei*. They attributed this action to adherence of *S. cerevisiae* cells to the gut and the secretion of amylase enzymes which shared in the increased digestibility of the diet.

On the other hand, the increased growth performance of *O. niloticus* treated with commercial products Megalo and Diamond-V yeast containing living *S. cerevisiae* with *B. subtilis* and dead *S. cerevisiae* respectively could be also attributed to the inhibition of some intestinal bacterial flora and increasing the non-specific immunity of the treated *O. niloticus*. The adherence capacity of *S. cerevisiae* and *B. subtilis* to the intestinal mucosa inhibits the attachment of the other intestinal bacteria to these binding sites and so preventing the disease

Table (3): Effects of experimental diet on the growth performance (g/fish) of Nile tilapia fingerlings

Items	Super Biobuds			Bio-yeast		
	(g)			(g)		
	0.5 (T ₁)	1.0 (T ₂)	1.5 (T ₃)	0.5 (T ₄)	1.0 (T ₅)	1.5 (T ₆)
Initial body weight	5.22 ± 0.03	5.22 ± 0.06	5.21 ± 0.05	5.17 ± 0.02	5.26 ± 0.02	14.5 ± 0.03
Final body weight	27.133 ± 0.9a	24.20 ± 1.26ab	22.76 ± 0.2bc	22.40 ± 1.03bc	25.43 ± 1.51ab	23.03 ± 0.36bc

A, B, C Means within row with different letters are significantly different (P<0.05).

occurrence with its negative impact on the fish growth (Thomas and Chhorn, 2011)

Physiological parameters

Data in Table (4&5) showed significant (P<0.05) increase in red blood cell count (RBCs), hemoglobin

(Hb) and hematocrit (Ht) in fish fed with a commercial probiotics under studied (Super Biobuds, Bioyeast and Stop stress gold) compared with the control. The same results were recorded by Jiri and Minarik (2003) who found that growth promoter tended to stimulate erythropoietin

Table (4): Effect of experimental diet on the condition factors (K) and survival rates (%) of Nile tilapia fingerlings.

Items	Super Biobuds			Bio-yeast			Stop stress gold			Control (T ₀)
	(g)			(g)			(g)			
	0.5 (T ₁)	1.0 (T ₂)	1.5 (T ₃)	0.5 (T ₄)	1.0 (T ₅)	1.5 (T ₆)	0.5 (T ₇)	1.0 (T ₈)	1.5 (T ₉)	
K	1.551 ± 0.059	1.449 ± 0.050	1.434 ± 0.175	1.397 ± 0.040	1.504 ± 0.038	1.407 ± 0.037	1.353 ± 0.028	1.333 ± 0.121	1.466 ± 0.083	1.254 ± 0.018
Survival rate %	A	AB	AB	AB	AB	AB	AB	AB	AB	B
	100	100	99	98	100	100	99	98	100	98

A, B, Means within row with different letters are significantly different (P<0.05).

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Table (3): Continued.

Items	Stop stress gold (g)			Control (T ₀)
	0.5 (T ₇)	0.5 (T ₇)	0.5 (T ₇)	
Initial body				
weight	5.18±0.04	5.18±0.04	5.18±0.04	5.21±0.08
Final body				
weight	21.57±0.60bc	21.57±0.60bc	21.57±0.60bc	21.43±0.47bc

A, B, C Means within row with different letters are significantly different (P<0.05).

which manifested itself as a higher erythrocyte count, a higher hematocrit level and higher hemoglobin concentration during the trial, compared with the control group. The increasing RBCs count, Hb and Ht values may be due to the enhancement of fish health. In the same Table (4) glucose showed slight difference, specially, in fish group fed with Bioyeast (0.05 & 0.1%) and Stop stress gold (0.15%). On the other hand, globulin showed without significant changes. No significant alternations occurred in the glucose levels except in fish group fed 0.05%, 0.1% of Bioyeast and 0.15% of Stop Stress Gold which may be due to handling mistakes (Table, 5). The ratios between albumin and globulin were significantly increased but T₁₀ had the lower value. Performing blood chemistry analytic determinations could provide vital information to aid in the diagnosis and management of infected individuals or in fish health assessment (Cnaani *et al.*, 2004 and Rehulka *et al.*, 2004). The

quantitative determination of the total protein reflects the liver capacity of protein synthesis and denotes the somnolently of the blood and the renal impairments. So it is of valuable effects in the diagnosis of the healthy status of fish (Marzouk *et al.*, 2008). The non – specific immune response parameters measured in the present study, i. e. Albumin and globulin concentration are commonly used for evaluation the effect of nutrients on the fish immunity. Measurement of globulin is of considerable diagnostic value in laboratory animals as it relates to general nutritional status, the integrity of the vascular system, and liver function. High albumin may result from increase of synthesis, or decrease catabolism (Nguyen *et al.*, 1999). The increase in albumin and globulin with addition of Super Biobuds, Bioyeast or Stop Stress Gold in fish diet may be due to the increase of their production in liver. Similar results were obtained by Ahmed *et al.*

Table (5): Survival rates (%) of Nile tilapia fingerlings challenged with *A. hydrophila*.

Items	Super Biobuds P ₁ (g)			Bio-yeast P ₂ (g)		
	0.5 (T ₁)	1.0 (T ₂)	1.5 (T ₃)	0.5 (T ₄)	1.0 (T ₅)	1.5 (T ₆)
Survival rates (%) after injection	94.44±0.6 A	83.33±0.6 AB	77.77±0.6 ABC	61.11±0.7 ABC	88.89±0.1 A	66.66±0.6 ABC

A, B, C,D..... Means within row with different letters are significantly different (P<0.05).

(2006) in case of selenium addition. The ratios between albumin and globulin were significantly increased due to the increase in albumin. Total lipids were fluctuated may be due to the fluctuations in energy demands. Data in Table (4) showed that AST, ALT, creatinine and urea were significantly decreased in all treated groups compared with the control group .In this study, the control fish showed high levels of these parameters

than any normal fish, so except that the addition of Super Biobuds, Bioyeast and Stop stress gold to the fish rations may be enhance the healthy status of the fish and their organs. Aspartate aminotranseferase (AST) and alanine aminotranseferase (ALT) enzymes as well as creatinine and urea are frequently used to diagnose the sub-lethal damage to certain organs; especially the liver and kidney (Benedeczky *et al.*, 1984).

Table (6): Effects of different probiotics on blood parameters of (*Oreochromis niloticus* Lin.) fingerlings.

Items	Super Biobuds P ₁ (g)			Bio-yeast P ₂ (g)		
	0.5 (T ₁)	1.0 (T ₂)	1.5 (T ₃)	0.5 (T ₄)	1.0 (T ₅)	1.5 (T ₆)
HB g/100ml	7.19±0.0a	6.58±0.09 b	6.30±0.10c	5.93± 0.10d	6.93±0.60a	6.19± 0.07cd
Hematocreat	22.12± 0.6a	15.34± 0.56bc	13.53±0.94cd	14.18±1.00bcd	20.43±.60 a	12.34±0.70de
HBT	2.44± 0.1a	1.88± 0.03b	1.76± 0.08bc	1.62± 0.08bc	2.41±0.16 a	1.75±0.06 bc
Creatinine g/100ml	0.35± 0.10g	0.56± 0.03e	0.64±0.03bd	0.76±0.02c	0.43±0.02 f	0.67± 0.02d
Urea g/100ml	8.70± 3.10g	10.94± 0.13e	13.83±0.34d	15.34± 0.28c	9.44± 0.30fg	15.38±0.33 c
Glucose g/100ml	56.35±3.10b	84.67±1.71a	75.64± 0.84b	67.16±3.30 c	62.73±3.06cd	77.75±1.33 b
AST µ/l	55.71±0.92g	68.08±1.17e	74.00±1.30d	82.65±1.71c	60.02±1.86fg	77.36±1.33d
ALT µ/l	22.72±1.59g	31.81±0.55d	32.27±0.91d	37.10±.082c	26.95±0.84e	34.15±1.33cd

A, B, C,D,E,F,G..... Means within row with different letters are significantly different (P<0.05).

PROBIOTICS AS GROWTH PROMOTER AND ANTI-BACTERIAL IN IMMUNE RESPONSE OF OREOCHROMIS NILOTICUS

Table (5): Continued .

Items	Stop stress gold P ₃ (g)			Control (T ₀)
	0.5 (T ₇)	1.0 (T ₈)	1.5 (T ₉)	
Survival rates (%) after injection	49.99±0.6 BCD	44.44±0.0 CD	88.88±0.5 A	22.22±0.5 D

A, B, C,D..... Means within row with different letters are significantly different (P<0.05).

Mukhopadhyay *et al.* (1982) reported that there is an intimate relationship between serum transaminases levels and liver integrity. Several factors affect the efficacy of probiotics on disease prevention in fish, especially the type of probionics and dietary dose concentration (dietary concentration + feeding duration). In tilapia, short-term feeding (2 weeks) and long-term (2 months or greater) have all proven to be effective in enhancing disease resistance in tilapia (Table, 5 included

as supplementary data). Few tilapia studies have explored the effects of dose concentration, although several have examined the effect of dietary concentration and feeding duration separately. Published information on immunostimulants suggests that, the larger the dose concentration, the less effective immunostimulants are in protecting fish against infection and can even result in immune suppression (Sakai, 1999 and Z.hou *et al.*, 2010)

Table (6): Continued.

Items	Stop stress gold P ₃ (g)			Control (T ₀)
	0.5 (T ₇)	1.0 (T ₈)	1.5 (T ₉)	
HB g/100ml	5.19±0.07f	5.09±0.09f	6.90±0.07a	5.54±0.19e
Hematocreat	11.12± 0.50e	13.31±0.80cde	16.08± 0.50b	12.26±0.93de
HBT	1.47± 0.08c	1.66± 0.07bc	2.33± 0.13a	1.13± 0.05d
Creatinine g/100ml	0.86± 0.01c	0.81± 0.01b	0.47± 0.01f	0.93±0.01a
Urea g/100ml	17.80±0.75b	16.60± 0.03bc	10.29±0.22ef	22.42±0.96a
Glucose g/100ml	74.60±2.09 b	76.68±2.54c	67.83± 1.43c	78.82±0.91ab
AST µ/l	93.09±2.23b	68.92±1.15c	64.10±1.77ef	109.9±1.07a
ALT µ/l	38.69±0.58ab	36.74±1.27bc	27.10±0.30e	41.18±1.28a

A, B, C,D,E,F,G..... Means within row with different letters are significantly different (P<0.05).

Table (7): Effects of different probiotics on plasma lipid and proteins of (*Oreochromis niloticus* Lin.) fingerlings fed experimental diets

Items	Super Biobuds P ₁ (g)			Bio-yeast P ₂ (g)		
	0.5 (T ₁)	1.0 (T ₂)	1.5 (T ₃)	0.5 (T ₄)	1.0 (T ₅)	1.5 (T ₆)
Total lipid g/%	6.90±0.35e	8.29±0.26bcd	8.66±0.18bc	9.69±0.29abc	7.37±0.58de	9.07±0.44ab
Total protein g/%	3.64± .25a	3.43± 0.11ab	3.38±0.11ab	2.94±0.04cd	3.44±0.05ab	3.12±0.05bc
Albumin g/dl	2.18± .02a	1.97± 0.0bc	1.83±0.07cd	1.61± 0.03ef	2.09±0.04ab	1.73±0.02de
Globulin g/dl	1.46±0.23ab	1.45± 0.11ab	1.55±0.05a	1.33±0.04 ab	1.35±0.04ab	1.39±0.04ab
A/G ratio*	1.58± 0.29a	1.37± 0.12a	1.18±0.04a	1.22± 0.05a	1.55±0.07a	1.25± 0.05a

A, B, C,D,E,F,G..... Means within row with different letters are significantly different (P<0.05).
Albumin/ Globulin

Effects of experimental diets on the mortality after injection by pathogenic bacteria (*A. hydrophila*)

The lowest value of K value was obtained by T₀ (control group). But, the differences between T₀, T₈, T₇, T₄, T₆, T₃, T₂ and T₅ groups were not significant (p>0.05). The values of growth coefficient (Table, 6) showed a linear observation was recorded by (Hernandez *et al.*, 1995) who mentioned that approximation to a linear trend in growth (constant growth rate) is indicated K values close to 1.0

where as departure from 1.0 mean either increasing rates or decreasing weight gain of *Penaeus styliarstris*. Results of injection by pathogenic bacteria of Nile tilapia are illustrated in Whereas, the value of survival rate was lowest in the T₀ (control group) than all groups (Table, 7). A result of the survival rate was higher in T₁ which fed on 0.05 % Super biobuds/kg diet of Nile tilapia. The survival rate in control group was 22.217 %. The probiotics supplementation has greater effects than diet without growth promoters in

Table (8): Effects of different probiotics in economical cost of the experimental diets

Items	Super Biobuds (g)			Bio-yeast (g)			Stop stress gold (g)			Control (T ₀)
	0.5 (T ₁)	1.0 (T ₂)	1.5 (T ₃)	0.5 (T ₄)	1.0 (T ₅)	1.5 (T ₆)	0.5 (T ₇)	1.0 (T ₈)	1.5 (T ₉)	
Cost/ton LE	2300	2360	2390	2330	2360	2390	2350	2400	2450	2300
Feed cost per kg weight gain	4.114	4.054	4.727	4.427	4.097	4.474	4.544	5.208	4.165	4.513
Feed cost/kg weigh gain %	90.76	89.23	104.29	97.66	90.38	98.70	100.25	114.89	91.88	100

PROBIOTICS AS GROWTH PROMOTER AND ANTI-BACTERIAL IN IMMUNE RESPONSE OF *OREOCHROMIS NILOTICUS*

Table (7): Continued

Items	Stop stress gold P ₃ (g)			Control (T ₀)
	0.5 (T ₇)	0.5 (T ₇)	0.5 (T ₇)	
Total lipid g/%	10.12±0.22a	9.74±0.16a	7.70±0.21cde	10.11±0.34a
Total protein g/%	2.54±.04ef	2.67±0.07de	3.37±0.08 ab	2.25± 0.06f
Albumin g/dl	1.45± 0.15f	1.45± 0.07f	2.02±0.02abc	0.88± 0.03g
Globulin g/dl	1.09± 0.17b	1.22± .10ab	1.36±0.07ab	1.38± .08ab
A/G ratio*	1.43± 0.39a	1.21± 0.14a	1.49± 0.05a	0.64±0.05b

A, B, C,D,E,F,G..... Means within row with different letters are significantly different (P<0.05). Albumin/ Globulin

improving live body weight and feed efficiency that increased the survival rates of fish. Probiotics were used for prophylaxis and treatment to eliminate or reduce bacterial contamination to a degree that enhances host defense mechanism. Fish under intensive culture conditions will be badly affected and often fall prey to different microbial pathogens that have been treated with chemotherapeutic substances of which antibiotics were intensively used. The use of natural immunostimulants in fish culture for the prevention of diseases is a promising new development and could solve the problem of massive antibiotic use (Jesus *et al.*, 2002). Further, they improved feed conversion ratio and utilization, revealed adhesion capacity to the intestinal mucosa that hindered the adherence of pathogenic bacteria, produced extra-cellular antibiotic like products or iron binding agents (siderophore) that prevent the growth

of some pathogenic flora. The probiotics fed fish groups showed high resistances to the challenged pathogenic microorganisms. Watson *et al.* (2008) demonstrated certain modes of probiotic action in the aquatic environment. In the present study, fish fed experimental diets revealed high resistances to the challenged pathogenic microorganisms Also the probiotic achieved improvement in water quality (bioremediation) and facing the problem of red tide planktons. From the immunostimulating point of view, many researches showed improvement in the immune response fishes treated with probiotics (Jesus *et al.*, 2002; Thomas *et al.*, 2011 and Zhou *et al.*, 2010).

Net profits of the feeding trial

Net partial budget analysis (Table, 8) was carried out to compare the profitability of using commercial probiotic in Nile tilapia diets, the total

costs of producing one ton of diet are shown in Table (8). The lowest cost was obtained by T₀ and T₁ diet followed by T₄ diet. The high costs of production of T₃, T₆ and T₉ diets according to the prices of probiotics included in this experimental diet. The relation between feed costs to produce one kilogram body weight gain was recorded also; the efficiency to get one Kg body weight gain was recorded in T₂ group followed by T₅, T₁ and T₉ groups. From economic point of view, it could be reported that using 0.10% Super Biobuds in Nile tilapia fingerlings diet had the highest economic efficiency being 89.235 %, while the lowest percentage was in T₈ group which had 0.1 % Stop stress gold / kg diet. Generally, yield increased by using probiotics while final fish weight and survival rate decreased in control group.

Results obtained could be summarized as the following

- 1- Using different probiotics in tilapia diets was superior to control diet.
- 2- The best results of growth performance were obtained by fish fed on diets supplemented with 0.05%, 0.10% and 0.15 % from Super Biobuds, Bioyeast and Stop stress gold, respectively.
- 3- There were significant increase in red blood cell counts, hemoglobin and hematocrit in tested fish fed diets supplemented with experimental probiotics.
- 4- The addition of probiotics in Nile tilapia diets enhanced the healthy status of the fish organs. Probiotics increased survival rate of fish compared with control group.
- 5- Using Super Biobuds at levels 0.1 % /kg of Nile tilapia fingerling diets have economically efficient. But, the differences were not significant at 0.05 %.
- 6- The best values of economic efficiency expressed of feed cost/Kg weight gain were recorded for diets containing 0.10 % Super Biobuds however control diet was the worst and recorded the lowest values on the growth performance, feed utilization and economic efficiency using probiotics as a feed additive aggregated effects than the control group which improving live body weight and feed efficiency as well as increased the economic efficiency of these diets.

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استخدام البروبيوتك كمنشطات نمو ومضاد بكتيري وتأثيرها على القياسات
الفيسيولوجية و الاستجابة المناعية لأصبعيات البلطي النيلي
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المُلخَص العربي

أستخدمت المضادات الحيوية قديما كمنشطات نمو فى بحوث الاستزراع السمكى وحديثا تم اختبار المنشطات الحيوية للقضاء على الامراض و تحسين مقاومة الجسم لها والهدف من الدراسة الحالية هو اختبار. تأثير ثلاث أنواع من البروبيوتيك المعروفة تجاريا مثل **Stop stress gold ، Bioyeast ، Super Biobuds** وذلك بمستويات ٠,٥ ، ١,٠ ، ١,٥ جرام/كجم علي معدل النمو ، القياسات الفسيولوجية ، الاستجابة المناعية والكفاءة الاقتصادية لعلائق أصبعيات البلطي النيلي. غذيت الاسماك بمعدل ٥% من وزن الجسم على أربعة وجبات متساوية على ٩ علائق تجريبية أضيفت بها ٣ مستويات مختلفة من البروبيوتك والعليقة العاشرة هى الكنترول بدون اضافات. فى نهاية التجربة قسمت الاسماك الى مجموعتين الاولى تضم ٩ معاملات و الاخرى تضم المجموعة الكنترول و تم حقنهم بالبكتريا المرضية وأخذت النتائج خلال أسبوعين لتقييم الاستجابة المناعية كذلك اجريت الاختبارات الفسيولوجية بأخذ عينات من الدم من مجموعتين من الاسماك.أوضحت النتائج فاعلية استخدام المنشطات الحيوية فى تحسين أداء النمو مع الاستجابة المناعية و أن التحسن فى زيادة النمو ترجع لزيادة البروتين و الجلوبيولين فى سيرم الدم و كذلك أثرت ايجابيا على الاستجابة المناعية غير المكتسبة .ونستخلص من الدراسة الحالية ان استخدام المنشطات الحيوية كأضافات غذائية فى علائق البلطي النيلي له تأثير ايجابى على النمو وربحية المزارع من خلال خفض تكلفة الاستزراع لانتاج وحدة الاسماك.

الكلمات الدالة: البلطي النيلي، البروبيوتك، القياسات الفسيولوجية وابحاث الاستجابة المناعية .