Assessment of some feed additives as immune promoters for *Oreochromis niloticus*.

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Abstract

This study was performed to compare between, Nosiheptide (antibiotic), ***Chlorella spp*** (algae), ***Lactobacillus plantarum (L. plantarum*** probiotic) and ***L. plantarum*** plus ***Azotobacter vielandii*** Exopolysacchride (***A. vielandii*** EPS) (prebiotics) as immune stimulators for ***Oreochromis niloticus*** fish. 150 ***O. niloticus*** (average body weight 50±2.3 g) were stocked randomly in 15 glass aquaria and divided into 5 groups each had 3 replicates, the 1st was the control group without any additives, the 2nd supplemented with Nosiheptide (3 g-kg diet), the 3rd was supplemented with ***Chlorella spp*** (10g/kg), the 4th were supplemented with ***L. plantarum***(10^{12} bacterial cells/ Kg feed) while the 5th were supplemented with ***L. plantarum*** plus ***Azotobacter vielandii (A. vielandii)*** EPS (0.5 g/ Kg feed). The feeding period lasted for 6 weeks. The best results of growth performance parameters namely food conversion ratio, total weight gain, average daily gain and relative weight gain were obtained by the group supplied by ***L. plantarum*** with ***A. vielandii*** EPS. There no significant differences in survival rate between different groups. RBCs count, HB, PCV, MCV, MCH, and MCHC values indicated that fish received Nosiheptide group had the worst results as compared with other treatments. The group supplied by ***L. plantarum*** plus ***A. vielandii*** EPS revealed the highest hepatosomatic index, spleenosomatic index, WBCs count and the best differential leuckocytic count indicated improving in health and immune status. Feeding with probiotics with prebiotics and ***Chlorella spp*** led to stimulation of phagocytic activity and serum bactericidal activities and raised plasma total protein. After stop feeding the additives by 0, 7 and 14 days, ***O. niloticus*** had challenged against ***Aeromonas hydrophila*** and mortality rate was recorded for 14 days after injection it was obvious that group fed on ***L. plantarum*** with ***A. vielandii*** EPS had the lowest mortality rate. The results showed the positive effect of the ***L. plantarum*** plus ***A. vielandii*** EPS on immune status of ***O. niloticus*** fish.

Key words: Nosiheptide, *Chlorella*, probiotics, *Azotobacter vielandii,* Expolysacchride, immune response, *Oreochromis niloticus* .

Introduction

The natural fisheries resources contributed 37% of the total national fish production, while aquaculture activities contributed 63% of total production. The production, progressively had become more subjected to many problems related to artificial feeding, water quality, management, pathogens and low immunity. The control of fish diseases requires the use of medicated feed supplemented with antibiotics. Antibiotics had some seriously adverse effects as emergence of multiple drug resistant bacteria and antibiotic residues in animal products (**Wary and Davies, 2004**). Therefore, using natural feed additives (probiotics, prebiotics and vitamins) improve the nutritional level of aquaculture animals and improve immunity of cultured animals (**Patterson and Burkholder, 2003**). Lactic acid bacteria (LAB) are among the most important probiotic microrganisms typically associated with gastrointestinal tract where they play beneficial effects as producing many kinds of metabolites, which might affect the other microbes in the fish mid gut (**Vijayabaskar and Somasundaram, 2008**) .The Prebiotics refers to a group of natural sugars that is resistant to digestion by fish but can exclusively be utilized by specific friendly beneficial bacteria or probiotic, stimulating their growth or activity, causing them to

outgrow their pathogenic competitors in the fish's gut (**Catherin *et al.*, 2006**). ***Chlorella sp.*** is one of the most common microalgae used widely in aquaculture and it contains high levels of protein, pigments, vitamins, minerals and unknown Chlorella growth factors **(Shubert *et al.*,1985)**. Antibiotic, Nosiheptide could improve the growth performance of Jade Perch by stimulating the villus of small intestine **(Ying and jun, 2006).** So this study was performed to compare between different immune stimulator and its effect on non-specific immune system of ***O.niloticus*** fish.

Material and methods

1- Addetivies:-

a-antibiotic: Nosiheptide belonging to the e series of thiopeptide produced by ESIGMA purchased from local market. Dosage 3mg/kg diet.

b-probiotic: ***Lactobacillus plantarum (L. plantarum)*** (10^{12} bacterial cells/ Kg feed).

c-prebiotic: ***Azotobacter vielandii*** Exopolysacchride ***(A. vielandii*** EPS) 0.5 gm was added to 1Kg feed. Probiotic and prebiotic were isolated from fish gut and prepared at Microbiology department. Faculty of Science, Tanta University.

d-algae: ***Chlorella spp*** 10g/kg of diet. Algae was kindly obtained from Botany department. Faculty of Science, Tanta University All feed additives were added to the surface of the feed via a represented oil suspension (sun flower oil).

2-Tested fish:- A total of 150 apparently healthy ***O. niloticus*** fish were collected from private fish farms Kafer El-Sheikh Governorate and previously acclimated in indoor tanks in full glass aquaria measuring (80 X 40 X 40 cm) and maintained in aerated dechlorinated fresh water at 25 ± 2 ^{o}C for 14 days. They seemed healthy and had a uniform size and weight with average body weight 50±2.3 gram.

3-Experimental feeding system design:- In this experiment a total number of 150 ***O. niloticus*** were divided equally into 5 groups, the 1st was the control group without any additives, the 2nd supplemented with Nosiheptide, the 3rd were supplemented with ***Chlorella spp***, the 4th were supplemented with ***L. plantarum*** while the 5th were supplemented with ***L. plantarum*** plus ***Azotobacter vielandii (A. vielandii)*** EPS. Diet was distributed twice daily, 6 days/week. The feeding duration longed for a period of 6 weeks. Every 7days, the fish in each aquarium were weighted and the amount of feed was 3% of biomass and corrected according to the new fish biomass.

Growth performance was calculated according to the following equations:-

a-Average daily gain (ADG) = (W1-W0)/T Where, W0 and W1 were the initial and final body weight per gram, and T is the number of days in the feeding experimental period. (**Castell and Tiews, 1980**)

b-Total weight gain (TG) = Wt_1-Wt_0 Where wt1 is the final body weight (g) and wt0 is the initial body weight (g) **(Castell and Tiews, 1980).**

c-Feed conversion ratio (FCR)= Feed intake (g)/ weight gain (g) Where, the weight gain is (the biomass of fish at the start + the biomass of the dead fish- the biomass of the fish at the end) **(Tacon, 1987)**

d-Relative Growth Rate (RGR %) = 100 (final weight–initial weight)/Initial weight.

f-Survival rate (SR %)= (No. of fish at end / No. of fish at the start) ×100**.**

4-Blood sample collection and Heamatogram:- Red blood cell (**RBCs**) and White blood cell (**WBCs**) counts were counted by haemocytometer according to **Stoskopf (1993).** Blood film was prepared according to the method described by **Lucky (1977)**. Differential leukocytic count was calculated according to **Schalm (1986)**. Blood hemoglobin (**Hb**) was assessed by cyanometahemoglobin method (**Drubkin, 1964)**. In addition, **M.C.V.** Mean Corpuscular Volume, **M.C.H.** Mean Corpuscular hemoglobin and **M.C.H.C.** Mean Corpuscular hemoglobin concentration were calculated according to the formula mentioned by **Dacie and lewis (1975)**.

M.C.V. = (PCV / RBCs) x 10 as m/mm^3.

M.C.H. =(HB content gm/100ml/ RBCs) x 10 as m/mm^3.

M.C.H.C. =(HB content gm/100ml / PCV) x100 as %.

The concentration of total protein (**TP**) **Weichsellbaum (1946**) and albumin (**Alb**) (**Doumas *et al.,* 1971**) were measured by colorimetric methods, while, globulin concentrations (**Glo**) were determined by subtracting thealbumin concentration from concentration of total protein albumin.

5-Immune response assay:-

a-Challenge test:- At the end of the experiment period (6weeks) of feeding and after (7and14 days) of stopping the feeding with feed additives a total number of 30 fish (5 fish from each treatment) were injected I/P with the pathogenic ***A. hydrophila*** which was kindly obtained from fish diseases department, animal health research institute (0.3 ml of 10^8cells/ml) according to **Schaperclaus *et al.*** (**1992**), the injected fishes were kept under observation for 14 day to record the mortality rate.

Mortality rate % = No. of death in specific period x 100 / Total population during that period

b-Bactericidal activity:- Serum bactericidal activity was done following the procedure of **Kajita *et al.* (1990)**. Equal volumes (100µl) of serum and bacterial suspension $2x10^8$ (CFU) were mixed and incubated for 1 h at 25 ^oC. Blank control was also prepared by replacing serum with sterile PBS. The mixture was then diluted with sterile PBS at a ratio 1 : 10. The serum-bacterial mixture (100µl) was plated in blood agar and plates were incubated for 24 h at 37 ^oC. The number of viable bacteria was determined by counting the colonies grown in nutrient agar plates.

c- Macrophage phagocyte indices:- Leukocytes isolation was performed according to the method described by **Faulmann *et al.* (1983)** and phagocytic activities were determined according to **Kawahara *et al.* (1991).** Blood was collected from the caudal vessels by syringe moisten with heparin (100 IU/ml). ***C. albicans*** was prepared as 24 hours old culture, the number of ***C. albicans*** cells was counted for obtaining the required concentration $1x10^6$ yeast cells/ml. Separated peripheral blood leucocytes were adjusted to a concentration of 2.5 x 10^6 vaible cells/ml, then to each I ml volume of blood leucocytes adjusted ***C.albicians*** suspension was added, then incubated in a incubator (CO2 5-10%) at 37 ^0C for one hour. Cover slips were stained with Giemsa stain. The phagocytic activity was calculated according to the following equations :

Phagocytic activity = No. of Ingesting phagocytes / total No. of phagocytes.

Phagocytic index = No. of ingested ***C. albicans*** cells / No. of Ingesting phagocytes.

d- Measuring of hepatosomatic index and spleenosomatic index:-

At the end of experimental period, 5 fish from each group were dissected and the viscera were exposed. The liver and spleen were taken and weighed after which the indices were calculated according to these equations:

Hepatosomatic index (HIS) = weight of the liver/fish body weight. (**Htun-hun, 1978**).

Spleenosomatic index (SSI) = weight of the spleen/fish body weight.

6-Statistical analysis:-

Statistical analysis was performed using the analysis of variance (ANOVA). Duncan's Multiple Range **Duncan (1955)** was used to determine differences among treatments mean at significance level of 0.05. All statistics were run on the computer using the SAS program (**SAS, 1998**).

Results and Discussion

1- Growth performance of *Oreochromis niloticus* fed different immune promoters.

Data in the table (1) showed growth performance namely DWG, FCR, TG, FI and RWG. It appears that the best growth significantly ($P<0.05$) was obtained with the group fed diet supplemented with ***L. plantarum*** and ***A. vinelandii*** EPS followed by those having a diet containing ***Chlorella spp*** then ***L. plantarum***

group, while group received Nosiheptide and control group were the lowest. Results obtained by addition of ***L. plantarum*** with ***A. vinelandii*** EPS could be explained by the findings of **Lara-Floers *et al.*** (**2003**) and **Venkat *et al.* (2004**) who stated that such improvement may be due to the creation of balanced microbial population in the intestinal tract of the fish and to the role of ***Lactobacillus*** in preventing the harmful bacteria which invade and populate in the digestive system improving nutrient absorption. Also **Fooks *and Gibson,*** (**2002**) recorded the ability of crude EPS to increase the colonization of ***L. plantarum*** in fish intestine by facilitating the metabolism and the growth of ***Lactobacillus*** in the lumen. **Sayeda, *et al.* (2011)** found that growth performance of ***O. niloticus*** was improved by supplementation diet with ***Azotobacter*** bacteria as probiotic (34.62% increase in SGR). **Kongnum and Hongpattarakere (2012)** isolated 202 strain of lactic acid bacteria from wild adult shrimp and identified as ***Lactobacillus plantarum*** MRO3.12 after 6 week of feeding trail ***L. vannamei*** exhibited significant differences ($p < 0.05$) on RGR and FCR. Also survival rate was improved 98.89% compared to the control group 68.89%. Concerning the results obtained by group fed ***Chlorella spp*** which showed improving in growth parameters **Zeinhom (2004)** agreed with these findings as he found that inclusion of algae in fish diets improved insignificantly ($P<0.05$) the FCR (2.33) and feed intake. Also similar results obtained by **Tartiel *et al.* (2008)** who mentioned that the average values of FCR increased significantly ($P<0.05$) with increasing ***Chlorella spp*** replacement from 0 to 50%. However, incorporation 50% algae replacement resulted in significant high value of FCR 2.03 ± 0.08 ***Chlorella spp***. While group fed on Nosipheptide obtained the lowest results. Different findings mentioned by **Ying and Xian-jun (2006)** as they decided that Nosiheptide could improve the growth performance of Jade Perch by stimulating the villus of small intestine. These different findings could be explained by the differences between fish species.

Data presented in the table (1) concerning survival rate showed insignificant differences between different groups. These results agreed with those obtained with **Nakagawa *et al.* (2007)** who recorded that survival rate of ayu fish received diet contained ***Chlorella spp*** had SR 100% compared with control 98.3%. These results could be explained by findings of **Nakagawa *et al.* (1984)** who conducted an experiment on tolerance of ayu fish to hypoxic condition. The ***Chlorella***-extract group was distinctly more tolerant of hypoxic conditions than the control group and mortality rate after the experiment was 25% in the control group but only 10% in the ***Chlorella***-extract group. Results concerning survival rate of probiotics. Moreover, **El-Ashram *et al.* (2008)** concluded that, probiotics (Biobuds) can improve body gain and survival rate, **Abdelhamid and Elkatan (2006)** found that dietary supplementation of ***Saccharomyces cerevisiae*** (Biobuds) slightly improved body weight gain but reduced the survival rate of tilapia fingerlings.

2- Leukogram of *O. niloticus* fed different immune promoters.

Both ***L. plantarum*** with ***A. vinelandii*** EPS and ***Chlorella spp*** groups had significantly ($p<0.05$) higher total leucocytes count than the Nosiheptide and control group as shown in table (2). Significant increase was recorded in the proportion of, Lymphocytes, Monocytes particularly in fish group having ***Chlorella spp*** (***L. plantarum*** with ***A. vinelandii*** EPS) followed by ***Chlorella spp*** while, both control and Nosiheptide treated fish groups showed the lowest values. These findings agreed with the findings obtained **Irianto and Austin (2003**) who mentioned that feeding with probiotic led to stimulation of cellular rather than humeral immunity increasing the number of erythrocytes, macrophages and lymphocytes after two weeks of feeding. Also **Sealey and Gatlin (2001)** reported supplementation diets of ***Cyprinus carpio*** with polysaccharides (prebiotics) had increased lymphocyte numbers. **Nakagawa *et al.* (2007)** agreed with the present finding concerning ***chlorella spp*** as they recognized that the preventive action ex-

erted by ***Chlorella***-extract against diseases might involve some internal barrier to infectious diseases, such as an inflammatory response and an increase in the number of phagocytes (neutrophil and monocytes). Also, **Hong-jun and Xiao-ting (2005)** agreed with the results concerning Nosiheptide group that they found that Nosiheptide had an inhibitory effects on immune organ and lymphocyte proliferation of peripheral blood.

3- Erythrogram and serum protien of *O. niloticus* fed different immune promoters.

Data presented in tables (2 and 3) showed that HB, PCV, MCV, MCH, MCHC, TP, albumin, globulin content and albumin globulin ratio in ***O. niloticus*** diet had improved significantly ($P<0.05$) by supplementation of ***L. plantarum*** with ***A. vinelandii*** EPS and ***Chlorella spp***. while Nosiheptide group and the control group showed the lowest results. These results agreed with the findings of **Al-Dohail *et al.* (2010)**. The results showed that the RBC, MCHC, MCH and MCV in ***C. gariepinus*** fish maintained on the probiotic diet were significantly higher than the groups fed non-probiotic diet.

Data concerning group fed on ***Chlorella spp*** the results agreed with the findings of **Nakagawa and Kasahara (1985)** who recorded that feeding on ***Chlorella spp*** with percentages 1% and 2% had improved PCV% and HB g/100 ml (41.9 and 43.0) and (9.24 and 8.86) respectively compared with control (40.9 and 8.8). Also they had obtained different results concerning TP g/100 ml and Alb g/100 ml had lower values (2.98 and 2.97) and (2.42 and 2.11) compared with control (3.2 and 2.58). While results concerning Nosiheptide could be explained by ***O. niloticus*** suffered from malabsorption as mentioned by **(Navratil *et al.*, 1998)** who stated that albumin makes up more than half of the total protein present in serum; a decrease in albumin synthesis is caused by intestinal malabsorption syndromes, and protein-calorie malnutrition.

4- Immunological studies on *O. niloticus* fed on immune promoters.

O. niloticus fed on feed additives and control group were challenged with pathogenic ***A. hydrophila*** at the end of feeding period and after stopping feeding by 7 and 14 days. In first 3 days motality rate was recorded. After injection, ***O. niloticus*** showed external lesions represented as skin darkening, focal hemorrhages at base of fins, diffuse hemorrhages under the skin and fin rot while internal lesions were hemorrhagic foci on the parietal surface of the liver with gall bladder distention.

Results presented in table (4) concerning pahgocytic assay (activity & index), hepatosomatic index, spleenosomatic index, challenge test against ***A. hydrophila***, and serum bactericidal presented showed that immuno status of ***O. niloticus*** fed on diet supplemented with (***L. plantarum***, with ***A. vinelandii*** EPS), ***Chlorella spp*** and ***L. plantarum*** had improved significantly ($P<0.05$) as compared with group received diet supplemented with Nosiheptide or with control group. These results agreed with findings of **Nikoskelainen *et al.*, 2003**) who mentioned that serum immunoglobulin levels were significantly raised after 1 week. These results showed that fish immune parameters were enhanced by using probiotic bacteria. Also, **Irianto and Austin, (2003**) stated that feeding with probiotic led to stimulation of cellular rather than humeral (serum of mucus antibody) immunity enhanced within two weeks of feeding.. Also **Sealey** and **Gatlin (2001)** reported that ***Cyprinus carpio*** immunostimulated with polysaccharides (prebiotic) showed macrophage activation, increased phagocytosis, increased lymphocyte numbers, increased serum immunoglobulins, and increased lysozyme. Moreover **Al-Dohail *et al.* (2010**) concluded, that ***Lactobacillus*** bacteria is useful as a probiotic agent in ***C. gariepinus*** against these pathogenic bacteria (***S. xylosus, A. hydrophila*** and ***S.agalactiae***). As spleen size of fish is widely used as a simple measurable immune parameter [**Taskinen and Kortet, 2002)** and also plays an important role in haematopoiesis, antigen degradation and antibody production processing (**Turner, 1994)** so the highest **SSI** recorded with ***O. niloticus*** fed on diet supplemented with (***L.***

plantarum, with ***A. vinelandii*** EPS) indicated enhanced immune status. Also findings of **Kongnum and Hongpattarakere (2012)** supported our results as they found that ten days after infection with ***V. harveyi*** (5.3–5.5 log CFU ml^{-1}), significant survival ($p < 0.05$) of 77% was observed in ***Lactobacillus plantarum*** supplemented shrimp, while only 67% survival was observed in the control. Findings of concerned group fed diet supplemented with ***Chlorella spp*** agreed with those found by **Nakagawa *et al.* (2007)** as they found that preventive action exerted by ***Chlorella***-extract against diseases might involve some internal barrier to infectious diseases, such as an inflammatory response and an increase in the number of phagocytes, rather than an immediate effect on the disease as an antibiotic substance. A well regulated metabolism and balanced body constituents, both dependent on dietary history, could provide disease resistance.

Hong-jun and Xiao-ting (2005) supported our results concerning Nosiheptide group that they claimed that Nosiheptide had the inhibitory effects on immune organs and lymphocyte proliferation of peripheral blood. However, **Chan *et al.* (2008)** found an opposite result concerning Nosiheptide supplementation as they claimed that addition of Nosiheptide at 2 mg·kg-1 level in the basal formulated diet promoted the immune functions and antioxidation of rainbow trout, which might result in high resistance to diseases. These different results may be explained by the differences between fish species.

It could be concluded that supplementation of ***O. niloticus*** diets with ***L. plantarum***, with ***A. vinelandii*** EPS isolated from fish or micro algae ***chlorella*** had improved growth performance, FCR, health status and non-specific immunty which made fish more resistant for infectious diseases. Effect on ***O. niloticus*** with ***L. plantarum***, with ***A. vinelandii*** EPS lasted for more periods than other additives. So it was recommended to use probiotics with prebiotic isolated from fish or ***Chlorella spp*** as immune stimulants in ***O. niloticus*** diets rather than using antibiotic for supplementation namely Nosiheptide which had immunosuppressant effect.

Table (1). Growth performance of ***Oreochromis niloticus*** fed different growth promoters (mean ± SE).

Item	IW	FW	ADG	TG	FCR	FI	RGR	SR
C	50.1 ^{a} ±1.37	70.73^{c} ±2.45	0.49^{c} ±0.04	20.7 ^{c} ±3.7	2.21^{a} ±0.1	45.65^{a} ±7.9	41.43^{b} ±8.7	86.7^{a} ±5.77
N	50.6 ^{a} ±0.56	68.73^{c} ±0.8	0.41^{c} ±0.02	18.13 ^{c} ±0.9	2.31^{a} ±0.19	41.9^{a} ±2.8	35.85^{b} ±2	83.3^{a} ±5.77
CL	50.4 ^{a} ±1.0	77.6^{b} ± 1.6	0.64^{b}± 0.04	27.23 ^{ab} ±1.1	1.71^{b} ± 0.1	46.5^{a} ±2.8	54.1^{a} ±2.2	86.7^{a} ±5.77
L	49.9 ^{a} ±0.5	76.27^{b} ±0.95	0.63^{b} ±0.01	26.5^{b} ±0.7	1.64b^{c} ±0.08	43.4^{a} ±1.6	52.9^{a} ±0.72	90^{a} ±10
L+A	50.2 ^{a} ±0.6	80.62^{a} ±0.7	0.73^{a} ±0.12	30.45 ^{a} ±1.3	1.53^{c} ±0.09	46.5^{a} ±2.5	60.73^{a} ±3.3	93.3^{a} ±5.77

Group with different letter within the same column are significantly different at $P< 0.05$. C = control, N = nosiheptide, CL = ***chlorella***, L = ***L. plantarum*** and L+A = ***L. plantarum*** + ***A. vielandii*** IW= Intial Weight, FW= Final Weight, ADG= Average Daily Gain, TG= Total weight Gain, FCR= Food Conversion Ratio, FI= Feed Intake, RGR=Relative Growth Rate and SR=Survival Rate.

Table (2). Haemogram of ***Oreochromis niloticus*** fed different growth promoters (mean ± SE).

Item	C	N	CL	L	L+A
RBC $X10^6$	1.64^{d} ±0.03	1.43^{c}±0.05	2.46^{a} ±0.07	2.2^{b} ±0.17	2.49^{a} ±0.1
WBCs $X10^3$	62.6^{bc} ±0.56	59.7^{c} ±0.86	64.8^{bc} ±1.9	65.31^{b} ±4.37	77.6^{a} ±3.7
H%	39.3^{bc}±2.9	52^{a}±2.6	34.7^{cd}±2.1	42^{b}±5.2	30.7^{d}±2.1
M%	2.67^{bc}±1.1	1.33^{c}±0.58	4^{ab}±1	2.67^{bc}±0.58	4.67^{a}±0.58
L%	55^{bc}±2.65	44.3^{d}±2.5	58.3^{ab} ±1.5	52^{c}±6	62.3^{a} ±0.58
E%	2.33^{a} ±1.5	2^{a} ±1	2.67^{a} ±1.1	2^{a} ±1	2^{a} ±1
B%	0.67^{a} ±0.58	0.33^{a} ±0.58	0.67^{a} ±0.58	1.33^{a} ±0.58	0.33^{a} ±0.58
HB (g/dl)	7.27^{c} ±0.15	5.43^{d} ±0.75	9.25^{ab} ±0.1	8.85^{b} ±0.4	9.64^{a} ±0.12
PCV %	18.33^{d}±0.4	14.23^{e}±0.81	27.03^{b}±0.42	25.6^{c}±0.26	30.4^{a}±1.1
MCV (m/mm^3)	111.6^{b}±1.5	99.4^{c}±6.8	110^{b}±1.7	116.2^{ab} ±8.5	122.4^{a}±5.14
MCH (m/mm^3)	44.2^{a}±7.2	37.9^{c}±6.4	37.4^{c}±0.57	40.2^{c}±2.1	38.7^{c}±1.2
MCHC (g/dl)	39.7^{a}±1.5	38.1^{a}±3.9	34.2^{b}±0.7	30.2^{b}±0.15	35.3^{c}±1

Table (3). Serum protein analyses of ***O. niloticus*** fed different growth promoters (mean ± SE).

Item	TP (g/100ml)	Albumin (g/100ml)	Globulin (g/100ml)	Alb/Glo
C	5^{c} ±0.26	2.73^{bc}±0.21	2.27^{b}±0.15	1.21^{ab} ±0.1
N	3.9^{d}±0.1	2.27^{c}±0.21	1.63^{c}±0.2	1.42^{a} ±0.2
CL	6.53^{b}±0.1	3.21^{b}±0.03	3.32^{a}±0.05	0.97^{b} ±0.03
L	6.33^{b}±0.05	3.1b±0.2	3.24^{a}±0.29	0.96^{b} ±0.2
L+A	7.66^{a}±0.2	4^{a}±0.3	3.7^{a}±0.5	1.11^{ab} ±0.07

Table (4). Immunological assay of ***Oreochromis niloticus*** fed different growth promoters (mean ± SE).

Item	P.I.	P.A.	HSI	SSI	Mortality rate			Serum bacteri cidal test(%)
					After 0 days	After 7 days	After 14 days	
C	1.87^{c} ±0.03	72.8^{c} ±2.9	1.35^{b} ±0.04	0.2^{b} ±0.06	76.7^{ab} ±1.58	0	0	46^{c} ±2.4
N	1.97^{b} ±0.4	69.3^{c} ±0.9	1.18^{c} ±0.05	0.18^{c} ±0.02	83.3^{a} ±1.58	0	0	37.77^{d} ±0.4
CL	2.5^{a} ±0.16	83.6^{a} ±2.3	1.72^{a} ±0.1	0.25^{a} ±0.05	50^{c} ±1.7	53.3^{a} ±3.5	66.6^{a} ±1.2	49.47^{a}b ±0.8
L	2.33^{ab} ±0.02	78.7^{b} ±3.6	1.48^{b} ±0.09	0.22^{b} ±0.01	46.7^{cb} ±3.5	66.6^{a} ±3.15	76.6^{a} ±2.5	47.9^{bc} ±1.5
L+A	2.68^{a} ±0.16	85.2^{a} ±2.14	1.78^{a} ±0.24	0.26^{a} ±0.02	53.3^{c} ±25	53.3^{a} ±2.1	60^{a} ±4.1	52^{a} ±1.5

Group with different letter within the same column are significantly different at P< 0.05. **C = control**, **N = nosiheptide**, **CL = *chlorella***, **L = *L. plantarum***, **L+A = *L. plantarum* + *A. vielandii***. **P.A.=Phagocytic assay** and **P.I.=Phagocytic index**.

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تقييم استعمال بعض أضافات الأغذية كرافع مناعي لأسماك البلطي النيلي المستزرعة.

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الملخص العربي

فى تجربة معملية لدراسة تأثيرالمضاد الحيوي (نوشيبتيد) وطحلب الكلوريلا و البروبيوتك (لاكتوبسيلاس بلانيترم) وبروبيوتك مضاف اليه بريبيونك (ازوتوباكتر فيلاندي) على رفع مناعة اسماك البلطي النيلي . تم تجميع 180 سمكة بلطي نيلي وزنها (حوالي 50 جرام) قسمت الى 6 مجموعة بإجمالى عدد 18 حوض زجاجى لكل حوض تهويته الخاصة. قسمت المجموعة الى عدد 3 مكررات وهى محفزات المناعة بتوزيعها السابق بالاضافة لمجموعة مقارنة (ضابطة) وإستمرت التجربة مدة 42 يوم.

وكانت أهم النتائج المتحصل عليها :-

1- أعلى نسبة نسبة تتحويل للاعلاف ومعدل اكتساب الوزن اليومي وزيادة الوزن الكلي و معدل اكتساب الوزن النسبي كانت للمجموعة المغذاة علي بروبيوتك مضاف اليه بريبيونك و المجموعة المغذاة علي طحلب الكلوريلا وكانت أقل النتائج بالمجموعة المغذاة علي المضاد الحيوي. لم يتم تسجيل اختلاف معنوي في معدلات الاعاشة بين المعاملات.

قدرة بروبيوتك مضاف اليه بريبيونك وطحلب الكلوريلا على رفع قدرة الخلايا البلعمية بدم الاسماك (النشاط الالتهامي).

3- كان اسوء النتائج المسجلة لصورة دم (عدد خلايا الدم الحمراء و عدد خلايا الم البيضاء و الهيموجلوبين و) اسماك البلطي النيلي في المجموعة المغذاة علي (نوشيبتيد) مقارنة بباقي المعاملات.

4- اختبار حساسية الاسماك للعدوي بيكتريا (الايروموناس هيدروفيلا) في نهاية التجربة وبعد انتهائها بـ 7 و 14 يوم كانت اقل في معدل نفوق الاسماك بالمجموعتين المغذتان علي بروبيوتك مضاف اليه بريبيونك وطحلب الكلوريلا.

5- كانت افضل النتائج الخاصة بـ (hepatosomatic index, spleenosomatic index) و(serum bactericidal activities) للمجموعة المغذاة علي بروبيوتك مضاف اليه بريبيونك وكذلك المجموعة المغذاة علي طحلب الكلوريلا.

توصي الدراسة بإستخدام البروبيوتك مضاف اليه بريبيوتك او الكلوريلا كرافع لمناعة اسماك البلطي النيلي مع تجنب استعمال المضاد الحيوي(نوشيبتيد).