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Evaluation of immune-modulatory effect of some probiotics in *Oreochromis niloticus*

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Abstract

Fish diseases that resulted from exposing to different microbial pathogens are intensively treated with antibiotics. Improved response against infectious diseases can be achieved by the use of probiotics as new alternative methods of disease control and to reduce the massive use of antibiotics. Evaluation of biochemical, immune-modulatory and pathological effect of dietary probiotics on *O. niloticus*has been studied. *O. niloticus* received a diet supplemented with a mixture of Lactobacillus and Pediococcus showed significant increase in the invitro phagocytic activity, serum lysozomal, bactericidal activity, total protein and globulin levels. Significant increase in antibody titration against *Aeromonas hydrophila* with significant decrease in serum albumin, albumin/globulin ratio, cortisol hormone levels and bacterial counts in gastrointestinal tract of *O. niloticus* received Lactobacillus & Pediococcus comparing with Saccharomyces cerevisce treated groupswas also detected. The histopathological examination confirmed that probiotics have protective effect to liver and kidney tissues under *Aeromonas hydrophila* infection. It could be concluded that Lactobacillus and Pediococcus are the better strains for aquaculture in this study. These strains provide a great potential to increasing the sustainability and efficiency of aquaculture production.

Key words: Bactericidal activity; Histopathology; Immunostimulants;*O. niloticus*;Probiotics.

Introduction

The intensive rearing of fish in aquaculture generates a potentially stressful environment to it, with suppression of the immune response rendering the fish more susceptible to different pathogens and diseases (Rohlenova and others 2011). The routine use of antibiotics during fish culture to minimize the risk of disease is not advisable since it may adversely affect the indigenous microflora of juveniles or adult fish and may increase the risk of promoting antibiotic- resistant microorganisms(Defoirdt and others 2011). Thus, the use of probiotics, in the past 10 years in the culture of aquatic organisms, is increasing with the demand for moreenvironment-friendly aquaculture practices to control diseases(Balcazar and others 2006).

Probiotics are members of the healthy intestinal microbiota. It is a live microbial feed supplement; therefore, they may provide an alternative way to reduce the use of antibiotics in aquaculture, since their addition can assist in returning a disturbed microbiota to its normal beneficial composition and able to provide a great balance in the microbial flora of the intestine(Brunt and others 2007; Mohapatra and others 2013). Probiotics utilize effectively the ingested food leading to acceleration the growth and production of fish (Reid and others 2003). Probiotics were used for enhancement of the immune response,growth performance and food utilization through improved intestinal health, (B and others 2014).Probiotics nowadays arepreferable health-promoting fish foodas they have prophylactic, therapeutic and growth promoter effect(Ouwehand and others

growth promoter effect(Ouwehand and others 2002). There are several mechanisms of action that have been reported for probiotic strains. They stimulate and augment the live weight gain and feed conversion efficiency, in fish, provide protection against different pathogens, production of hydrogen peroxide, organic acids and lysozyme (Son and others 2009). Moreover, they stimulate fish immune response and used as a biological control agents in intensive highly stocked ponds of aquaculture(Morya and others 2013; Nimrat and others 2012). Probiotics are a nature effective product in aquaculture have potential effective role on immunity, intestinal ecosystem as well as fish health, performance and production. Probiotics efficacy may be potentiated by several ways via the selection of more efficient strains, gene manipulation and the combination of several strains(Isolauri and others 2004). This bacterial strain mixture approach seems to be one of the best methods of potentiating the probiotics efficacy and is widely used in practice.It has much potential to increasing the aquaculture production. Therefore, our research highlights effect of two important commercialprobioticson health and immune status in *O. niloticus*.

Material and methods

2.1. Probiotics:

Two commercial products (probiotics) have been used in this study to evaluate their effects.

. The first product named C.A. growth® and contains a mixture of Lactobacillus *acidophilus* $1x10^9$ CFU/ml & Pediococcus $1x10^9$ CFU/ml.

The second product named tonolest® and contains *Saccharomyces cerevisce* $15x10^9$ cells/gm active live yeast.

2.2. Fish and maintenance:

Two hundred and twenty fiveculturedNile tilapia (*Oreochromis niloticus*), with an average body weight of (50±5g/fish). Fish were purchased from Baraseek Aquaculture fish farm, at Albehera Governorate, Egypt. Fish were kept in triplicate throughout the experimental period in a glass aquaria (90×50×35 cm), supplied with 80 liters chlorine free areated tap water.Fishwere acclimated for two weeks before starting the experiment. The water temperature was kept at 25-28℃ using heater (CMI, Germany). The PH was 8.3 ± 0.3 and was monitored with a PH meter. Fish were fed on a commercial fish diet containing 25% crude protein twice a day at rate 4 % of their body weight.Water quality parameters contained the following concentrations: 144.5 ± 8 mg $CaCo_3$ /l total hardness, 8.2± 0.3 ppm dissolved oxygen, 0.01mg/ l Nitrite (No_2), 0.02 mg /l Nitrate (No_3), 20± 7 mg/ l H_2s and 0.01 mg/l un-ionized ammonia. Two thirds of water was changed daily.

2.3. Experimental design

Two hundred and twenty five cultured Nile tilapia were divided into five groups each consists of three aquaria (triplicate), the experiment was carried out for seven weeks.

Group I (n=45), negative control, fish were fed on basal diet only, supplemented with clean water and left without any treatment along the experimental period.

Group II(n=45), fish were fed on basal diet contains a probiotic mixture of Lactobacillus & Pediococcus (0.1mL/ kg feed).

Group III(n=45), fish were fed on basal diet contains a probiotic a mixture of Lactobacillus & Pediococcus (0.2 mL / kg feed).

Group IV(n=45), fish were fed on basal diet contains *Saccharomyces cerevisce* as a probiotic (0.15 g/ kg feed).

Group V(n=45), fed on basal diet contains *Saccharomyces cerevisce* as a probiotic (0.3 g/ kg feed).

The study was carried out at the Faculty of Veterinary Medicine, Alexandria University, Egypt. The experimental procedures were complied with the Animal Welfare Act and approved by the Alexandria University's Veterinary Committee.

sterile needles and syringe. Blood was collected with syringe containing anticoagulant (0.1 ml of 4% sodium citrate solution/1ml blood) and used for phagocytic assay according to (Wilson 1990). Blood was allowed to flow smoothly into a clean glass tubes and left to clot for 2 hours at room temperature then centrifuged (BOECO centrifuge, Germany) at 3000 rpm for 15 min. The clear supernatant serum was collected using sterile Pasteur pipettes (Sigma-Aldrich). The collected serum was transferred to dry, sterile labeled tubes and used for different biochemical assays.

2.5. *Phagocytic activity and phagocytic index*

Phagocytic activity was determined according to (Wilson 1990).*Candida albicans* was kindly supplied by the department of poultry and fish diseases Faculty of Veterinary Medicine, Alexandria University.
Phagocytic activity (PA) = percentage of phagocytic cells containing yeast cells.

$$\text{Phagocytic index (PI)} = \frac{\text{Number of yeast cells phagocytized}}{\text{Number of phagocytic cells}}$$

2.6. *Estimation of biochemical parameters:*

Serum proteins and albumin were colometrically determined according to (Busher 1990 andKrohn 2002)using commercial kits provided by Pasteur, Lab, France. Serum globulin level was determined by subtracting the albumin from the total proteins according to (Busher 1990),where A/G ratio was estimated using albumin and globulin values. Determination of cortisol level (Morineau and others 1997) using commercial radioimmunoassay (RIA) kits

2.4. *Blood sampling:*

Blood samples were collected at the end of the experiment. Benzocaine solution (1 g/10 L) was used to anesthetize fish. Blood was collected from the caudal vessels using

provided by IBL, GmbH, Hamburg, Germany. Serum lysozyme activity was measured using turbidimetric method described by(Morsky 1983). Lyophilized *Micrococcus lysodekticus* (Sigma M 3770) were used for the serum lysozomal activity.The result was expressed as one unit of lysozyme activity was defined as a reduction in absorbency of 0.001/min.

2.7. *Determination of serum bactericidal activity against Aeromonas hydrophila:*

The Strain was kindly supplied by the dept. of poult. and fish dis., Faculty of Vet. Med., Alexandria University. It is used for the serum bactericidal activity study was determined according to (Vasudeva Rao and others 2006). In sterile ependorf tubes, 300 ul of *A. hydrophila* suspension (1.5 x 103 cells / ml) and 300ml of fresh serum were mixed. A blank consisted of 300 ul of bacterial suspension and 300 ul of sterile phosphate buffer saline (PBS) then the tubes were incubated at 28 C. Then removed 50 ul sample at 0, 1, 2, 3, 4h for plated different dilutions on nutrient agar for 24 h at 28 C, and colony forming units (CFU) were counted. The results were recorded as survival index (SI)

$$SI = \frac{\text{CFU at end}}{\text{CFU at start}} \times 100.$$

2.8. *Challenge and post challenge protection test:*

At the end of 7th week, ten fish from all groups were clinically examined. Blood samples were bacteriologically tested and determined to be free from bacterial infection. Collected fish were then artificially

infected by intraperitoneal injection (IP) with 0.2 ml of culture suspension of pathogenic *A. hydrophila* adjusted to $1X10^4$ CFU/ml. The specificity of death was confirmed by re-isolation of injected bacteria from freshly determined according to (Ruangpan and others 1986)using the following equation.

$$RLP = 100 - \frac{\text{Percentage of immunized mortality}}{\text{Percentage of control mortality}} \times 100$$

$$\text{Mortality \%} = \frac{\text{NO of death in a specified period}}{\text{Total population during that period}}$$

2.9. Histopathological studies:

Liver, kidney and spleen were harvested from all groups. Tissues were immediately prepared for hisopathological examination according to (Suvarna and others 2013).

2.10. Statistical analysis

Data were collected and analyzed by one-way analysis of variance ANOVA (Bewick and others 2004) to estimate the effect of different treated groups on different parameters. Data were presented as mean ± SE and significance was declared where different letters are significantly different.

Results

3.1. Effect of probiotics treatments on phagocytic activity and phagocytic index:

Significant ($P \leq 0.05$) increase in the phagocytic activity and phagocytic index were detected in groups II & III when compared with the control group. Significant ($P \leq 0.05$) increase in the phagocytic activity was detected in groups V with non-significant changes in group IV. While groups IV & V showed significant ($P \leq 0.05$) increase in the phagocytic index comparing with control (Table 1).

3.2. Effect of probiotics treatments on total proteins, albumin, globulin and albumin/globulin ratio:

dead fish during the period of observation (One week) according to (Langdon and others 1985). The relative level of protection (RLP), among the challenged fish was

Serum total proteins and globulin were significantly ($P \leq 0.05$) increase in all treated groups with significant ($P \leq 0.05$) decrease in the albumin/globulin ratio compared with the control. Groups II & III revealed a significant ($P \leq 0.05$) decrease in the serum albumin meanwhile groups IV & V showed a non significant change comparing with control (Table 2).

3.3. Effect of probiotics treatments on cortisol level:

All treated groups showed a significant decrease in the cortisol level compared with the control group. Groups IV & V showed significant ($P \leq 0.05$) increase in the cortisol compared with other treated groups (groups II and III), (Table 3).

3.4. Effect of probiotics treatments on serum lysozyme & bactericidal activity.

The serum lysozyme was significantly ($P \leq 0.05$) increased in group III comparing with other groups. Meanwhile, the bactericidal activity was significantly ($P \leq 0.05$) increased in the groups II & III comparing with other groups (Table 4).

3.5. Effect of probiotics on relative level of protection (RLP) and mortality after challenge with A. hydrophila

The mortality rate was increased in groups IV & V comparing with other groups. Meanwhile, the relative level of protection (RLP) showed higher level in C groups II & III (70% in group III and 50% in group II) comparing with other groups (Table 5).

3.6. Histopathology:

The histopathological findings *O. niloticus* fed on basal diet incorporated with Lactobacillus & Pediococcus (groups II &III) revealed no marked difference in the

microscopic picture. *O. niloticus*treated with large dose of probiotics mixture (group III) showed minimal changes than the group treated with small doses (group II). The liver of groups II, III &IV, showed moderate hydropic degeneration with normal nucleus and congestion in blood vessels and activation of kupffer cells (Fig. 1A,B&C). *O. niloticus* treated with large dose of Saccharomyces cerevisce (0.3ml/kg feed, group V),*O. niloticus*challenged with *A. hydrophila* and fed on dietincorporated withC.A growth and *O. niloticus*challenged with *A. hydrophila* and fed on dietincorporated withTonolest showed sinusoidal congestion, moderate fatty change and congestion in central vein(Fig. 1D, E &F). The kidney of groups II & III (treated with 0.1 & 0.2 ml/kg feed of C.A growth® probiotic), showed vaculation in proximal convoluted tubules, congestion in interstitial blood vessels, hydropic degeneration in epithelium and with glomerular atrophy and dilatation of bowman's space (Fig. 2A&B). *O. niloticus* treated with (0.15 &0.3 ml/ kg feedTonolest® probiotic (groups IV &V), showed severe vaculation in epithelium with pyknosis in nucleus, necrosis in tubules and interstitial edema (Fig. 2C), meanwhile *O. niloticus*challenged with *A. hydrophila* and fed on dietincorporated withC.A growth, showed vaculation in proximal convoluted tubules, glomerular atrophy and congestion in interstitial tubules (Fig. 2D).

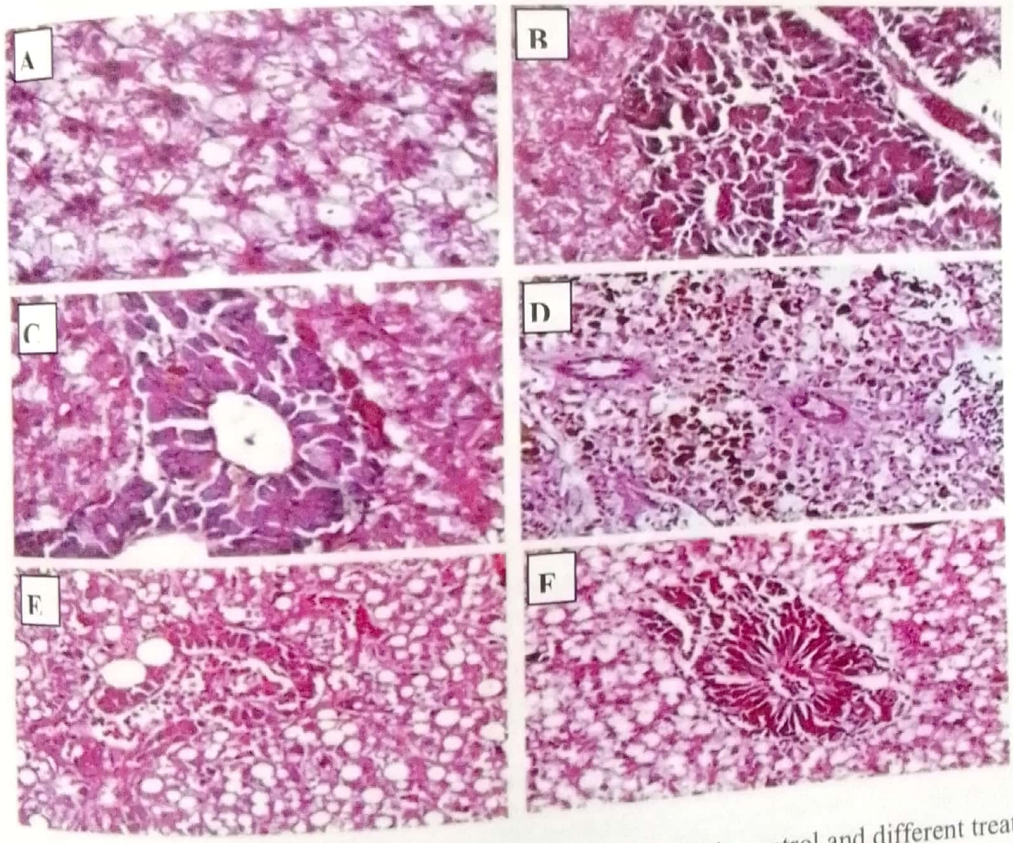

Fig. (1): Histopathological findings in liver of *O. niloticus*in control and different treated groups:

Histological analysis of the liver from the different experimental groups (original magnification ×400), (**A**) Group II, showed moderate hydropic degeneration; (**B**) Group III, showed moderate hydropic degeneration and congestion in blood vessels; (**C**) Group IV, showed moderate hydropic degeneration with mild congestion; (**D**) Group V, showed moderate fatty change and congestion in central vein; (**E**) *O. niloticus*challenged with *A. hydrophila* and fed on dietincorporated witha probiotic

mixture of Lactobacillus & Pediococcus (0.1mL/ kg feed)., showed sinusoidal congestion, moderate fatty change and congestion in central vein; (**F**) *O. niloticus*challenged with *A. hydrophila* and fed on dietincorporated with*Saccharomyces cerevisce*, showed moderate fatty change, sinusoidal congestion, congestion in central vein and the remaining cells suffered from hydropic degeneration.

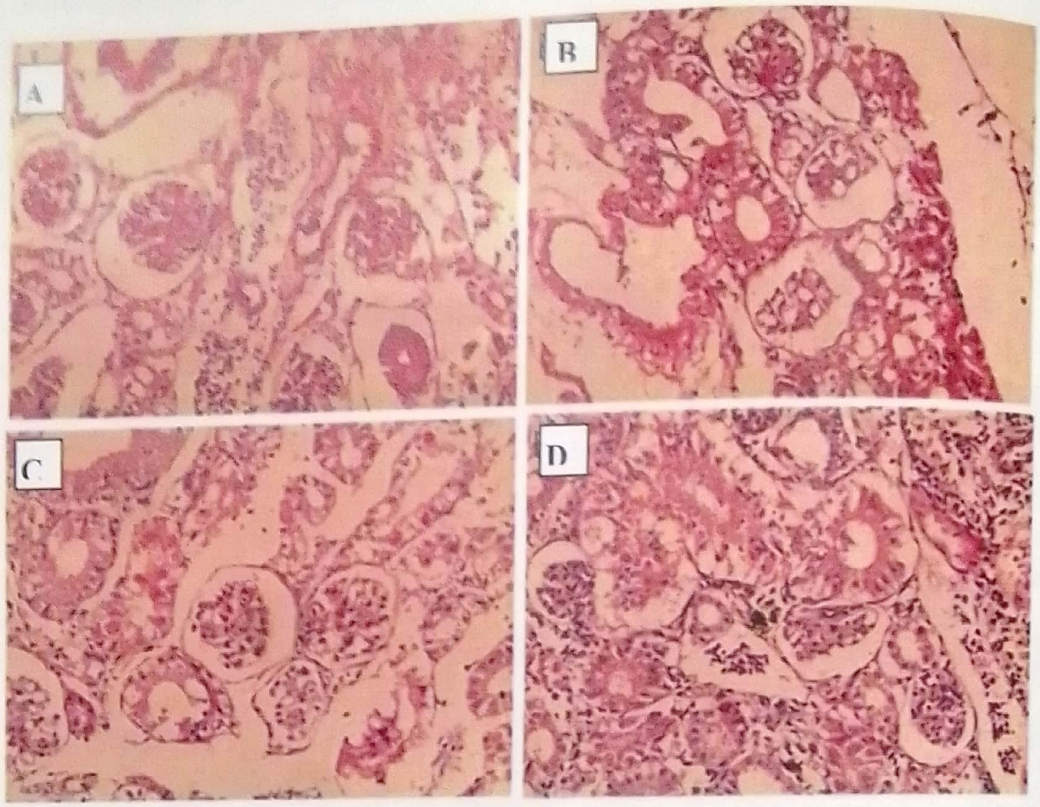

Fig. (2): Histopathological findings in kidney of *O. niloticus*in control and different treated groups.

Histological analysis of the kidney from the different experimental groups (original magnification ×200), (**A**) Group II, showed vaculation of proximal convoluted tubules, dilatation of Bowman's space due to glomerular atrophy and interstitial edema; (**B**) Group III, showed vaculation in tubular epithelium with glomerular atrophy and dilatation of bowman's space; (**C**) Group IV, showed necrosis in proximal convoluted tubules (eosinophilic area) and hyaline droplet formation; (**D**) *O. niloticus*challenged with *A. hydrophila* and fed on dietincorporated with C.A growth, showed vaculation in proximal convoluted tubules, glomerular atrophy and congestion in interstitial tubules.

Table (1): Showing results of phagocytic activity and phagocytic index among control and treated groups.

Groups	Phagocytic activity	Phagocytic index
Group I	21.00±0.58^{e}	2.27±0.09^{d}
Group II	30.67±0.33^{b}	3.27±0.09^{b}
Group III	38.67±1.20^{a}	4.07±0.09^{a}
Group IV	25.00±0.00^{c}	2.53±0.09^{c}
Group V	23.00±0.58^{d}	2.47±0.03^{cd}

Values represent the mean± SE of five samples for each group.
Values in the column with different superscript letters are significantly different at $P \leq 0.05$.

Table (2): Showing results of serum total protein, albumin, globulin and albumin/globulin ratio among control and treated groups.

Groups	Total protein	Albumin	Globulin	A/G ratio
Group I	4.40±0.30^{d}	3.00±0.10^{a}	1.40±0.40^{d}	2.84±1.24^{a}
Group II	7.50±0.15^{b}	2.13±0.12^{b}	5.37±0.24^{b}	0.40±0.04^{b}
Group III	7.97±0.12^{a}	1.83±0.07^{c}	6.13±0.09^{a}	0.30±0.01^{b}
Group IV	5.47±0.09^{c}	2.80±0.06^{a}	2.67±0.07^{c}	1.05±0.03^{b}
Group V	5.03±0.12^{c}	2.87±0.12^{a}	2.17±0.24^{c}	1.37±0.23^{b}

Values represent the mean±SE of five samples for each group.
Values in the column with different superscript letters are significantly different at P≤0.05

Table (3): Showing results of cortisol level among control and treated groups.

Groups	Cortisol level
Group I	548.40±4.04^{a}
Group II	423.90±4.33^{c}
Group III	404.00±2.57^{d}
Group IV	507.70±2.47^{b}
Group V	513.40±2.36^{b}

Values represent the mean± S.E. of five samples for each group.
Values in the column with different superscript letters are significantly different at P≤0.05

Table (4): Showing results of serum lysozyme and bactericidal activityamong control and treated groups.

Groups	Serum lysozyme	Bactericidal activity
Group I	0.01±0.00^{b}	3.70±0.15^{d}
Group II	0.07±0.01^{b}	5.27±0.20^{b}
Group III	0.17±0.02^{a}	6.30±0.29^{a}
Group IV	0.05±0.01^{b}	4.23±0.09^{c}
Group V	0.04±0.01^{b}	4.07±0.09^{c}

Values represent the mean± SE of five samples for each group. Values in the column with different superscript letters are significantly different at P≤0.05

Groups	RLP	Mortalities

Table (5): Showing mortality percent and relative level of protection after challenge with pathogenic bacteria *(A. hydrophila)* among control and treated groups.

	No	%	No	%
Group I	0	0	10	100
Group II	5	50	5	50
Group III	7	70	3	30
Group IV	2	20	8	80
Group V	2	20	8	80

Discussion

Fish culture is rising on wide scales to compensate the needs for animal proteins, as the production of fish is increasing tremendously allover the world; the needs for new strategies for disease control are evoked. This study was planned to evoke the differential aspects of using commercial probiotics in *Oreochromis niloticus* from their effects on the immune response of treated fish to pathogenic bacteria, changes in gut microbiota and cortisol hormone level point of view as well as the histopathological studies of treated fish.

Probiotics can effectively trigger the pahgocytic cells in host and enhancement of phagocytic activity by LAB group of probiotics such as *L. rhamnosus, L. lactis* and *L.acidophilus*(Gill and Guarner 2004). Fish received Lactobacillus and Pediococcus

(groups II &III) showed a significant increase in phagocytic activity and phagocytic index than groups IV & V (received diet supplemented with Saccharomyces cerevisce 0.15 & 0.3 gm / kg feed). These results are directly proportional with the result of histological examination and this explain that the groups treated with (C.A. growth®) probiotic showed stimulation in the non-specific immune response than *O. niloticus*that received (Tonolest®) probiotic. Contrary to our findings, the yeast act as an immunostimulant by stimulating the immune response via increasing the phagocytic activity, respiratory burst activity(Butprom and others 2013). In our study the combination between bacteria and yeast in (C.A. growth®) probiotic give good results in phagocytic assay than using the yeast type only in (Tonolest®) probiotic.

Changes in the physiological state often reflect alteration of serum biochemical parameters. C.A. growth® probiotic treated fish showed a significant increase in serum total protein and globulin level, meanwhile albumin, A/G ratio and plasma cotisol showed a significant decrease comparing with groups IV &V (Tonolest® probiotic treated fish). The improvement of fish health when fed *L. acidophilus* supplement diets could be attributed to the immuno-modulatory effect of *L. acidophilus*on the liver cells which activate the anabolic capacity of the hepatocytes to produce blood proteins particularly globulin (Harikrishnan and others 2011). Our histopathological examination revealed that treated fish with large dose (0.2 ml/kg C.A. growth®) probiotic exhibited no marked pathological alterations as the liver showed moderate hydropic degeneration and congestion in blood vessels and activation of kupffer cells. The serum total protein was significantly improved in fish maintained on the diet supplemented with the probiotic, *L. acidophilus*. Albumin/ globulin ratio is a measurable humeral component at the non-specific defenses. The reduction of A/G ratio might be due to the increase of total serum globulin level with significance protective mechanisms for fish (Sahoo and Mukherjee 2001). Contrary oral administration of Baker's Yeast (S .cerevisiae) stimulate the non specific immunity level as measured through enhanced phagocytic activity and

reduced A/G ratio which appeared with (Tonolest®) probiotic treated groups (Patr 2011). Our histopathological examination of liver in group V received large dose (0.3gm/ kg feed Tonolest®) probiotic showed fatty change in which the hepatic cell converted into fat cell in which the cytoplasm filled with fat and the nucleus pouched to the peripheryand this explain the lower value of serum protein and globulin levels have been reported due to impaired liver functions which synthesized plasma protein.

The level of cortisol was decreased when fish was fed a diet supplemented with *L.fructivorans* and *L.plantarum* (Abelli and others 2009). Lysozyme, one of the important bactericidal enzymes of innate immunity is an indispensable tool of fish to fight against infectious agents (Mai and others 2014). Fish received the high dose of C.A. growth® probiotic (0.2ml/kg feed) (group III) showed a significant increase in serum lysozyme and bactericidal activity comparing with other groups. The combination in vivo between *B. subtilis* and *L. acidophilus* is able to enhance the bactericidal and lysozyme activity(Aly and others 2008). The increase in serum bactericidal activity of *O.niloticus*against pathogenic bacteria may be due toeither the antimicrobial substances that produced by *L. acidophilus* or may be due to the increased natural complement, serum peroxidase or phagocytic activities (Havixbeck and others 2014). Th eincrease in the phagocytic, lysozyme and bactericidal activity of the head kidney macrophages of rainbow trout fed *C. maltaromaticum* and *C. divergenscompared*. Meanwhile, the viable bacterial counts showed a significant decrease was also reported (Kim and Austin 2006).

Concerning the challenge of the *O. niloticus* fish groups The results indicated thatthe groups treated with 0.1 and 0.2ml/kg feed (C.A. growth®) (groups II&III) showed high level of protection (50% and 70% respectively) and survival than the groups treated with (Tonolest®) probiotic (20% relative level of protection in both doses). These results confirmed the immunostimulatory effect of the living *lactobacillus* and *Pediococus* which present in (C.A. growth®) probiotic and also their inhibitory effect to *A. hydrophila*. The histopathological examination of liver of*O. niloticus* challenged experimentally with *A. hydrophila* and fed on diet incorporated with (C.A. growth®) showed sinusoidal congestion, moderate fatty change and congestion in central vein and the kidney showed vaculation in proximal convoluted tubules, glomerular atrophy and congestion in interstitial tubules and these pathological changes considered minimal changes than infected non treated groups.

These results confirmed the immune stimulatory effect of the living *S. crevisiae* and *B. subtilis* and also their inhibitory effect to *P. fluorscens* also the variation in the mortality ratios in both groups indicated that the living yeast and bacteria cells are more potent than dead yeast cell in the protecting the *P. fluorscens* infection.

It was not possible to discriminate the contribution of the yeast among the effects observed on water quality, growth, survival, immune response, or stress and disease resistance. However, the microorganisms might work synergistically in such consortia, and that may be worth further investigating.

Conflict of interest: The authors declare that there are no conflicts of interest.

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الملخص العربي

الكثير من مزارعي الاسماك يعتادوا استخدام المضادات الحيويه بكثافة وذلك لعلاج الامراض او كإضافات للعلائق لمنع حدوث الامراض. وحيث ان الاستخدام المفرط لتلك المضادات الحيوية مضر علي صحه ومناعه الاسماك وايضا ارتفاع اسعارها جعل اتجاهنا الي ايجاد بدائل لها . وفي هذا البحث تم عمل التجارب المعمليه باستخدام البروبيوتك كوقاية للأمراض وكذلك للحد من الاستخدام المفرط للمضادات الحيويه . وفي هذه الدراسه اجريت لتوضيح تاثير البروبيوتك علي الحاله المناعيه لاسماك البلطي وتوضيح مدي قدرته علي زياده قدره الاسماك البلطي المناعيه علي مواجهه العدوي بميكروب الايروموناس هيدروفيلا . خلال هذه التجربه تم احضار عدد 225 سمكه من اسمكا البلطي النيلي بتموسط وزن 5 ± 50 جم من مزارع بمحافظه البحيره . تم تقسيم الاسماك الي 5 مجموعات وكل مجموعه تحتوي علي 3 تكرارات . غذيت الأسماك علي خمس علائق لمدة 7 اسابيع.كانت المعاملات كالتالي: 1- عليقة ضابطة, 2- عليقة ضابطة أضيف إليها اللاكاتوباسلس والبيدوكوكس (0.1 مل / كيلوجرام عليقة), 3- عليقة ضابطة أضيف إليها اللاكاتوباسلس والبيدوكوكس (0.2 مل / كيلوجرام عليقة) و 4- عليقة ضابطة أضيف إليها سكارومايسس سيرفسس (0.15جرام / كيلوجرام عليقة). 5- عليقة ضابطة أضيف إليها سكارومايسس سيرفسس (0.3جرام / كيلوجرام عليقة). في نهاية التجربة تم اختيار 5 اسماك عشوائيا من كل مجموعة للحصول على الدم لقياس نسبه الليزوزيم وايضا الاجسام المناعيه .

ويمكن إيجاز أهم النتائج فيما يلى:

1-استخدام خليط اللاكاتوباسلس والبيدوكوكس ادي الي زياده معنويه في الحاله المناعيه وايضا ارتفاع في نسبه الليزوزيم وكذلك القدره المناعيه للاسماك علي مقاومه العدوي البكتيريه لاسماك البلطي النيلي .وايضا زياده في نسبه الاجسام المناعيه المقاومه للايروموناس هيدروفيلا . ايضا استخدام اللاكاتوباسلس والبيدوكوكس مقارنه بسكارومايسس سيرفسس ادي الي تقليل عدد البكتيريا الموجوده في الجهاز الهضمي للاسماك المستخدمه .

2- باجراء الدراسات الهستوباثولجيه للاسماك المستخدمه وجد التاثير الايجابي لاستخدام البروبيوتك في عمل حمايه للكبد والكلي لاسماك البلطي النيلي المستخدمه ضد العدوي بمرض الايروموناس هيدروفيلا .

3- خلال هذا البحث وجد ان خليط اللاكاتوباسلس والبيدوكوكس مقارنه بسكارومايسس سيرفسس ادي الي نتائج ايجابيه علي الحاله الصحيه والمناعيه الاسماك البلطي النيلي .