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INFLUENCE OF PROBIOTIC SUPPLEMNTATION ON BACTERIOLOGICAL, BIOCHEMICAL, MEAT, QUALITY PATHOLOGICAL AND GROWTH PERFORMANCE OF TILAPIA FISH EXPERIMENTALLY INFECTED WITH *PSEUDOMONAS AERUGINOSA.*

By

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ABESTRACT

The present study aimed to assess the impact of probiotic ABM (Activated Beneficial Micro-organisms) by1Liter/10m^{3}water on bacteriological, immunological aspects, growth performance, water and fish meat quality and pathological changes of tilapia fish against oxidative stress damage induced by *P. aeruginosa*. A lab trial was conducted for two months using 130 tilapia fish 15-20g.were divided into four equal groups (out of 10 fish were examined bacteriologically to ensure that they were free from *P. aeruginosa* and were stocked in four glass aquaria (30 fish per group).

All fish were acclimatized for three weeks. G1: kept as control negative, G2: was infected intraperitonially with (0.1×10^8cfu/ml) P. *aeruginosa* after 21 days from the beginning of the trial. , G3: was given probiotic ABM from the beginning of the experiment till the end, G4: subjected to add probiotic ABM from the first day till the end of the trial and infected with *P. aeruginosa* as mentioned before after 21 days from the beginning of the trial. Results revealed that addition of ABM in water aquaria lowered the level of ammonia, increased the dissolved oxygen concentration and growth performance. Body weight of fish showed a significant increase in group fed on ABM and also an improvement of organoleptic properties of tilapia and there were a significant decrease in total bacterial, psychotropic and Enterobacteriaceae count .In the same aspect addition of ABM achieved an improvement of TBA&TVN than control and infected group, protein and fat % within the permissible limits in treated groups, and . Significantly decrease of % *P. Aeruginosa* count and mortality rate in group was treated with ABM. GXP, GST and SOD they were improved and showed an

increase in their levels in groups that take ABM. Lysozyme activity and nitric oxide assay were significantly elevated in *P. aeruginosa* inoculated group. Meanwhile, the administration of ABM modulated the toxic effect of the bacterial infection, Total serum protein; albumin immunoglobulin showed a significant increase in group fed on ABM with bacterial inoculation. Moreover, albumin and immunoglobulin levels revealed significant increase in group fed on ABM alone which may be due to its immune stimulator and modularity effect. The finding of the present study supports the use of probiotic as immunostimulant in common fish diets. Results of histopathological examination revealed that fish infected with *P. aeruginosa* showed different pathological changes in gills, skin, muscles, liver, kidneys and gonads.

Keywords:

Probiotic-Growth-Tilapia fish-*P. aeruginosa*.

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INTRODUCTION

Tilapia fish is the most cultivated species, representing more than 65% of the total aquaculture production in Egypt **(Dickson *et al*., 2016).**

The excessive and inappropriate use of antimicrobial drugs, pesticides, disinfectants, and chemicals in disease prevention and growth promotion has led to drug resistance in Tilapia fish. To solve these problems, many environmentally friendly biological controls of bacteriological disorders have been developed and methods such as the use of probiotic have become an important subject of investigations **(Seddik *et al*., 2017).**Recent studies have been made to relieve stress and enhance immunity in aquatic animals using various feed additives in the diet, including vitamins, immune stimulants, prebiotics and probiotics **(Dawood *et al*., 2015).** Modulation of immune system is one of the most commonly reported benefits of the probiotics and their potency to stimulate the systemic and local immunity . Different probiotics supplementation can stimulate immune response as lysozyme ,phagocytic activity and gut immune system of fish with marked increase in immunoglobulins **(Doan *et al*.,2017).**

Total immunoglobulin showed significantly increased levels for fish supplemented with probiotic in addition to highest levels of lysozyme activity. These results support the use of probiotics as immunostimulants in common fish diets. **(Magda *et al*., 2011).**

Probiotic supplementation to fish diets can influence total serum protein, albumin and globulins significantly in addition to serum lysozyme activity **(Sayed *et al*., 2011).** Probiotics

have been used to improve the growth performance and decrease production costs of farmed tilapia in many studies **(Hai,2015),** also considered as safe alternatives to antibiotics, with several beneficial effects to the aquaculture industry **(Banerjee and Ray, 2017)** via different mechanisms such as competitive inhibition of pathogenic bacteria through the production of inhibitory compounds**,** enhancement of digestive enzymes activities which increase the availability of nutrients to the host, improvement of water quality and enhancement of immune and stress responses of fish **(Ibrahim, 2015).**

Probiotics are a live microbial adjuvant which has a beneficial effect on the host by modifying the host associated or ambient microbial community, by ensuring an improved use of the feed or enhancing its nutritional value, by increasing the host response towards disease, or by improving the quality of its environment **(Verschoor *et al.*, 2000)**.

Prebiotics are defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of specific health-promoting bacteria, which can improve host's health **(Gibson *et al*., 2003).**

Symbiotic are nutritional supplements that combine probiotics and prebiotics, enhancing their beneficial effects **(Cerezuela *et al*., 2011).**

Activated Beneficial Micro-organisms (ABM) product reduce the organic matter and ammonia concentrations inside the fish ponds to improve water quality for better growth and good fish health. All the constituents of ABM are produced naturally and does not include any genetically modified components or bacteriophage. Several reports have highlighted that probiotics including *bacillus subtilis, lichenformis* provide a more favorable environment for fish through reducing the proliferation of pathogenic bacteria and harmful phytoplankton as well as via the bioremediation of organic wastes in rearing water **(Banerjee and Ray, 2017).**

Reactive oxygen species (ROS) can result in oxidative stress (OS), which decreases body performance, accelerates aging, and causes a variety of diseases .A free radical scavenging system ensures that free radical generation and elimination are in dynamic balance to prevent oxidative damage to the body **(Tang *et al*.,2018).**

PCR allows rapid and complete identification of *P.aeruginosa* **(Gholami *et al*., 2016).** This method also allows the determination of resistance and virulence genes associated with this bacterium. *P. aeruginosa* produces several virulent factors to colonize the cells of its host. Many of these factors are controlled by regulatory systems involving cell-to-cell signaling

(Van Delden and Iglewski, 1998). Among these are: exotoxin A, Las B elastase. Exotoxin A, encoded by the *tox* A gene, inhibits protein biosynthesis by transferring an ADP- ribosyl moiety to elongation factor 2 of eukaryotic cells. The ability of *P. aeruginosa* to cause infection is further exacerbated by a high level of resistance to antibiotics which makes *Pseudomonas* infections difficult to be treated **(Hancock and Speert, 2000).** LasBelastase, a zinc metalloprotease encoded by the *las* B gene, attacks eukaryotic proteins such as collagen and elastin, and destroys the structural proteins of the cell **(Toder *et al.*, 1994).** Therefore the present study aimed to investigate the impact of adding probiotic (ABM) in the farming of Tilapia fish as a diet enhancer on quality of tilapia fish meat, and increasing in protein and fat ratio in muscle of fish. Natural immunostimulants are valuable for activating the fish immune system and protecting fish against adverse conditions **(Sakai *et al.*, 2001).**

The evaluation was based on the effect of immune response, possible protective effects against a challenge infection with *P.aeruginosa*, survival rate and growth performance.

MATERIAL AND METHODS

1- Probiotic:

Activated Beneficial Micro-organisms (ABM) consists of beneficial living organisms and yeast in addition to some safe plant extracts to increase fish immunity. ABM Composed of *Lactobacillu*s spp, .*Bacillus subtilis* and *Bacillus lichen forms*., Yeast extract (*Saccharomyces cerevisiae*),5 Safe plant extracts, dipotassium hydro phosphate, calcium Carbonate and natural colors.(ABM produced by the Aquatic Biology Research Department at the National Research Center and is registered for a patent No. 935 in 2018 and is currently being registered as a biological fertilizer).

2- Challenging bacteria:

P.aeruginosa strain (obtained from Reference Laboratory for Food Safety, Animal Health Research Institute, Doki, Egypt). The culture was diluted to achieve an inoculum level of approximately 0.1×10^8 cfu/ml.

3-Experimental design:

A total of 130, apparently healthy of Tilapia fish (15-20 g.) were obtained from Kafr El-Sheikh Kitchener. Ten randomly fish were examined bacteriologically to ensure that they are free from *P.aeruginosa* and the remains (120 fish) were divided into four groups in four glass aquaria (60x40x40 cm) (30 fish in each group) with three replicates . They were maintained in aerated, dechlorinated tap water. The temperature was adjusted at 26^{o}C as

well as continuous oxygen supply by air pump. Tilapia fish were fed twice a day with commercial pellet feed from Alaraqua company at a ratio of 4% of fish weight at the beginning of the experiment, 3% in the second month **(Mabrouk *et al.*, 2018).**The quantity of feed related to aquarium fish weight was adjusted through weekly weighing at early morning before feeding and the fish mortality was recorded daily (Table 2) and so, the quantity of feed was decided. Fecal matters were siphoned out once daily and third of aquarium water was changed daily to maintain a good water quality.

Tilapia fish were acclimatized for three weeks before the beginning of the experiment.

The groups were divided as follow:

1-The first group: Kept as negative control which was non infected group.

2-The second group: The fish was infected with 0.1×10^{8}(CFU/ml/fish) *P.aeruginosa* intra peritoneal (I/P) which represent control positive group.

3-The Third group**:** The fish was given in water ABM probiotic (1L /$10m^3$ water weekly) from the beginning of the experiment till the end (the period of the experiment is two month).

4-The fourth group: The fish was given ABM probiotic as mentioned before in addition to infection with *P. aeruginosa*0.1×10^{8}(CFU/ml. IP) at the 21^{st} day of the experiment.

The experimentally infected fish were inspected daily post-infection, the clinical signs, and necropsy finding were record.

Methods of evaluation of fish groups:

1-The mortality percentage was calculated weekly for each group.

2-Growth Performance and feed Utilization:

2.1. Body weight gain/g = Final fish weight (g) - Initial Fish weight (g) **(Annet, 1985).**

2.2. Feed Conversion Ratio (FCR) =Total feed Consumed by fish (g) /Total weight gain: by fish (g). **(Pouomong and Mbonglang, 1993).**

2.3. Protein Efficiency Ratio (PER) = Weight gain per Fish (g)/ Protein intake per fish (g)

3- Aquarium water Analysis:

3.1. Measurement of Water Temperature was done daily using a mercury Thermometer.

3.2. Dissolved Oxygen are measured daily using LaMotte – DO Tracer -1761.

3.3. Total Ammonia: are measured daily using (LaMotte, Ammonia -Nitrogen 0-2 ppm Code 3304 - 02).

3.4. Measurement of pH Value: Using pH meter (HI 8014, HANNA Instrument Portugal).

4- Blood Chemistry analysis of The Fish:

At the end of experiment blood samples were obtained from the caudal vein, at 4^{o}C for 24 hr. vertically and then centrifuged at 4000 rpm for 15 min. The supernatant was collected in 1.5 ml Eppendorf tube and immediately stored in a refrigerator at -20 ^{o}C for the following tests.

4.1. Total serum Cholesterol: It was determined according to **(Allain *et al.*, 1974)** using kit of Quamina Clinical Applicate S.A. (QCA).

4.2. Serum Urea and Serum Creatinine: It was carried out according to **(Crouch, 1997).**

4.3. Glutathione Peroxidase activity (GSH-Px): It is indirectly estimated according to **(Wendel, 1981).**

4.4. Glutathione-S-transferase activity(GST):The GST activitywasdetermined spectrophotometrically with aromatic substrate (CDNB) as described by **(Habig *et al.*, 1974).**

4.5. Superoxide dismutase activity (SOD): SOD activity was determined with an enzymatic assay method using a reagent kit (Randox, Crumlin, UK), as described by **(Sun *et al*., 2010).**

5-Keeping quality of the Tilapia fish and its nutritional value:

Collection of samples:

5.1. Organoleptic Evaluation of Whole Raw Fish: Number of raw whole tilapia fish collected for organoleptic evaluation. Sensory evaluation of raw tilapia fish were performed by ten trained panelists chosen from the staff members of the Animal Health Research Institute, Tanta Lab. The organoleptic assessment of raw tilapia was made according to the scale described by **(Barile *et al*., 1985).** This scale ranged from zero (extremely unacceptable) to 10 (highly acceptable) for the following characteristics: acceptability, eye, gills, body surface (appearance), odor, texture, flesh condition, viscera and belly wall**.**

5.2. Fish sampels for Bacterial and Nutritional Evaluation: Fish meat samples were divided into four parts; the first part of samples was collected under complete hygienic aseptic condition for bacteriological examination, the second part for determination of pH, the third part of samples for determination of protein and fat % and fourth part for chemical analysis.

5.2.1. Total Bacterial Count. CFU/g. was According to **(FDA, 2001).**

5.2.2. Total Psychotropic count. CFU/g. According to **(FDA, 2001).**

5.2.3. Total Enterobacteriaceae counts CFU /g according to **(Anonymous, 1991).**

5.2.4. *P.aeruginosa* count and its reduction% in fish meat samples.

5.2.5. Isolation and identification of some food poisoning microorganisms.

5.2.5.1. Total *S.aureus* count **CFU/g (APHA, 2001).**

5.2.5.2. Isolation of *Salmonella* **(ISO 6579, 2002).**

5.2.5.3. Isolation of *E.coli*. **(Quinn *et al.*, 2011).**

5.3. Nutritional evaluation of fish muscle.

5.3.1. Protein percent of the fish by Kjeldahl process as described by **(AOAC, 2000).**

5.3.2. Fat percent of fish muscle as described by **(AOAC, 2000).**

5.3.3. Total Volatile Basic Nitrogen (TVN) of fish muscle (mg /100 g) **(Ronald *et al.*, 1991).**

5.3.4. Thiobarbituric acid value (TBA) of fish muscle (mg/100g) according to **(Papastergiadis *et al.*, 2012).**

5.4. PH Value of fish muscle **(AOAC, 2000).**

6 - <u>Bacteriological Challenge:</u>

In the present study, a total of 180 samples by (180 samples from 60 fish by 3 samples from each fish) were collected from Tilapia fish muscles, gills and livers at five time during experiment at fourth, fifth, sixth, Seventh and eighth weeks for *P. aeruginosa* reisolation.

6.1. Bacterial culture and identification of *P.aeruginosa* **(APHA, 2002).**

Microscopical examination **(APHA, 1992)** and biochemical characterization of the isolate were performed according to the criteria of **(Murray *et al.*, 1984).**

6.2. PCR Procedure. DNA extraction. DNA extraction from samples was performed using the QIAamp DNA Mini kit(Qiagen, Germany,GmbH)with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56^{O}C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate.The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit. Oligonucleotide Primer. Primers used were supplied from Metabion (Germany) are listed in (Table 1).PCR amplification. Primers were utilized in a 25- µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an applied biosystem 2720 thermal cycler.

Analysis of the PCR products. The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µl of the products were loaded in each gel slot. Gel

pilot 100 bp plus ladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software**. (Matar *et al.*, 2002; Finnan *et al.*, 2004; Spilker *et al.*, 2004 and Winstanley *et al.*, 2005).**

Table (1): Primers sequences, target genes, amplicon sizes and cycling conditions.

Target gene	Primers sequences	Amplified segment (bp)	Primary Denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
toxA	F-GACAACGCCCTCAGCATCACCAGC R-CGCTGGCCCATTCGCTCCAGCGCT	396	94°C 5 min.	94°C 30 sec.	55°C 40 sec.	72°C 40 sec.	72°C 10 min.	Matar *et al.*, 2002
lasB	F-ACAGGTAGAACGCACGGTTG R-GATCGACGTGTCCAAACTCC	1220	1220	94°C 5 min.	94°C 30 sec.	54°C 40 sec.	72°C 12 min.	72°CFinnan *et al.* (2004)
exoS	F-GCGAGGTCAGCAGAGTATCG R-TTCGGCGTCACTGTGGATGC	118	94°C 5 min.	94°C 30 sec.	55°C 30 sec.	72°C 30 sec.	72°C 7 min.	Winstanley *et al.*, 2005
P. aerugi nosa16 SrDNA	F-GGGGGATCTTCGGACCTCA R-TCCTTAGAGTGCCCACCCG	956	94°C 5 min.	94°C 30 sec.	52°C 40 sec.	72°C 50 sec.	72°C 10 min.	Spilker *et al.*, 2004

7- Immunological Evaluation of fish:

7.1. Lysozyme activity assay.

Serum lysozyme activity was assessed by turbidimetric assay according to **(Ellis, 1990).**

7.2. Nitric oxide assay:

Serum nitric oxide (N.O.) level was estimated according to **(Yang *et al.*, 2010).**

7.3. Serum Total Protein (T. P.): Total protein concentration in serum was measured as described by **(Bakerman, 1984).**

7.4. Electrophoretic pattern of serum protein estimation: According to **(Sonnen Wirth and Jaret, 1980)** and calculated according Syn Gene S. No. 17292* 14518 sme* mpcs.

7.5. Immunoglobulin determination by zinc sulphate turbidity according to **(McEwan, *et al.*, 1970).**

8- Pathological examination:

8.1. Post mortem examination of fish was performed and the internal organs were carefully examined.

8.2. Specimens from gills,skin ,muscles liver, kidneys and gonads were fixed in 10% buffered formalin and processed for paraffin sections (3-5 µm thick) which was stained with Hematoxylin and Eosin (H & E) stain for histopathological examination according to **(Bancroft and Gamble, 2002).**

9-Statistical analysis:

All data were analyzed by GLM procedure of SAS 9.1 **(SAS Institute, 2003).** Dennett's Test was used to compare the means. All statements of significance were based on $p < 0.05$.

RESULTS AND DISCUSSION

Table (2): Mortality rate of examined fishes during experimental period.

Group No	No of dead fish										Total	
	W4						W5	W6	W7	W8	NO	%
	Zero day	1st day	2nd day	3rd day	4th day	5th day						
G_1	1	0	1	2	0	1	1	0	0	1	7	23.3
G_2	0	5	4	3	3	3	2	2	2	2	26	86.6
G_3	0	0	0	0	0	0	0	0	0	0	0	0
G_4	0	0	1	1	0	0	0	0	0	0	2	6.6

Zero days: day of infection with *P. aeruginosa*.

The percentage were calculated according to total number of each group (n=30).

Table (2) revealed cumulative mortalities of Tilapia fish during experimental period were 7 fish (23.3%) in group (G1) which fed on the basal diet only. The high mortality rate (86.6%) occurred in group (G2) which infected with *P. aeruginosa* without treatment.

These results may be attributed to the production of extracellular enzymes and toxins by *P.aeruginosa.* That results relatively agree with **(Abou El-Geit *et al*., 2013),** who stated that *P. aeruginosa* produces several virulent factors to colonize the cells of its host. Many of these factors are controlled by regulatory systems involving cell-to-cell signaling **(Van Delden and**

Iglewski, 1998). Among these are*:* exotoxin A, Las B elastase. Exotoxin A, encoded by the tox A gene, inhibits protein biosynthesis by transferring an ADP- ribosyl moiety to elongation factor 2 of eukaryotic cells. The same table revealed no mortalities rate of Tilapia fish were recorded in G3 which fed on probiotic from beginning to the end of experiment which mortalities were very rare with percentage of 6.6% in group (G4) which was pretreated with probiotic and infected with *P.aeruginosa* on the 21st day of the trial. *Bacillus* can displace pathogenic bacteria from the gut and accordingly enhance disease resistance and improve fish performance **(Hostins *et al.*, 2017).**

Table (3): Growth performance of Tilipia fish at the end of experimental period.

Items	Groups of experimental fish			
	G1	G2	G3	G4
Initial weight (g)/fish	30±0.57 [a]	29.1±0.72 [a]	30±0.60 [a]	29±0.57 [a]
Final weight(g)/fish	67±1.15 [c]	55.7±0.88 [d]	98.7 ± 2.03[a]	85.3±2.6 [b]
Weight gain (g)/fish	37±1[c]	26.5±0.76 [d]	68.5± 1.44 [a]	56.3± 1.45 [b]
FCR	2.06±0.06[b]	2.34± 0.07 [a]	1.35±0.03 [d]	1.6± 0.06 [c]
PER	1.48±0.02 [a]	1.06±0.08 [b]	2.68±0.02 [c]	2.24±0.07[d]

Results are presented as means ± standard error, n = 3.

The values with different superscript letters in a row are significantly different (p<0.05).

The results in (Table 3) revealed that, the final fish weight, weight gain, and protein efficiency ratio (PER) were improved with addition of ABM in G3& G4 versus to control group.

These results agree with **(Mabrouk *et al.*, 2018).** FCR in ABM treated groups was lower ($P < 0.05$) as compared with control .This attributed to the improved digestive activity due to enhancing the synthesis of vitamins and enzymatic activities **(Soltan and El-Laithy, 2016),** consequently, improving digestibility and growth performance. Since the first use of probiotics in aquaculture, a growing number of studies have demonstrated their ability to increase the growth rate and welfare of farmed aquatic animals **(Wang, 2007).** *Saccharomyces cerevisiae* (baker' yeast) is a good model of these organisms in both whole cell or extract forms. Benefit wise, such yeast is non-pathogenic, resistant to bile and acidic pH. **(Abu-Elala *et al.*, 2013).** Dietary *S. cerviceae*has been efficient in improving the growth performance and feed efficiency rate in *Oreochromis niloticus* **(He *et al.*, 2009).** Yeast cell, has been reported to produce some energy substrates for intestinal cells,which contribute to healthy gut **(El-Sayyad *et al.*, 2010).**

Table (4): Effect of ABM on water aquaria.

Parameters	G1	G2	G3	G4
Ammonia(mg/L)	**1.23±0.15^{b}**	**2.08±0.22^{a}**	**0.13±0.01^{c}**	**0.27±0.07^{c}**
Unionized Ammonia(mg/L)	**0.0074±0.0008^{b}**	**0.0125±0.0013^{a}**	**0.0052±0.00008^{c}**	**0.0016±0.0002^{d}**
pH	**6.8±0.21^{a}**	**7.47±0.15^{a}**	**5.5±0.29^{b}**	**7±0.29^{a}**
Temp. (℃)	**26±1**	**26±1**	**26±1**	**26±1**
DO (mg/l)	**4.04±0.18^{b}**	**3.48±0.3^{b}**	**5.65±0.18^{a}**	**5.44±0.2^{a}**

DO = dissolved oxygen.

Results are presented as means ± standard error, n = 3.

The values with different superscript letters in a row are significantly different (p<0.05).

In this study, total ammonia and non ionized form of ammonia are shown in (Table 4) were significantly lower ($p < 0.05$) in G3& G 4 than control and marked increase in ammonia and pH in water in G2 (infected.).The highest DO levels was observed in groups (G3&4) treated with ABM in water.

These contribute to improving water quality consequently fish health and may be attributed to the enhanced growth of beneficial bacteria. Accordingly, bacteria shed with fish excreta might change the bacterial community in favor of water quality improvement **(Balcázar *et al.*, 2006).**Values of water quality parameters reported in this study were within the range desirable for tilapia farming as reported by **(Mabrouk *et al*., 2018).**

Total ammonia nitrogen (TAN) is composed of toxic (un-ionized) ammonia (NH_3) and nontoxic (ionized) ammonia (NH_4) .The proportion of TAN in the toxic form increases as the temperature and pH of the water increase in G2. For every pH increase of one unit, the amount of toxic un-ionized ammonia increases about 10 times. Chronic exposure to toxic un-ionized ammonia levels as low as 0.06 mg/L (ppm) can cause gill and kidney damage, reduction in growth, possible brain malfunction, and reduction in the oxygen-carrying capacity of the fish**. (Robert *et al.,* 1997)**. In addition, TAN levels may be reduced due to higher oxygen levels in G3&G4 due to the positive effect of probiotic ABM on water fish aquarium also we noted that aquarium water was not changed and remain a good water quality of glass aquaria of G3 and still fit for 42 days . So we recommending to adding ABM

substance to fish tanks where it reduce effort of water replacing and consuming as well as we expect superior results for farmer due to large farming space also sun and fresh air exposure enhances the results drastically and we can conclude that ABM solving the most problems facing intensive fish farms and have a high economic value.

Table (5): Effects of ABM on serum total cholesterol, urea and creatinine onantioxidant enzymes activity (GPx, GST, SOD), in blood serum of Tilapia fish (Mean ± SE).

Groups Parameter	Cholesterol (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	GPX (u/ml)	GST (u/ml)	SOD (u/ml)
G1	123.67 ± 1.76 ^{b}	6.16 ± 0.09 ^{a}	0.76 ± 0.02 ^{ab}	33.81 ± 0.16^{b}	73.43 ± 0.31 ^{b}	48.10 ± 0.11 ^{b}
G2	141.00± 0.57 ^{c}	8.68 ± 0.31 ^{b}	0.91 ± 0.01 ^{c}	21.34 ± 0.29^{a}	60.93 ± 0.07 ^{a}	46.24 ± 0.59 ^{a}
G3	83.07 ± 0.20 ^{a}	5.99 ± 0.13 ^{a}	0.70 ± 0.01 ^{a}	52.33± 0.67^{d}	87.99 ± 0.14 ^{d}	93.37 ± 0.33 ^{d}
G4	126.67± 0.67 ^{b}	6.49 ^{x}± 0.07 ^{a}	0.80 ± 0.01 ^{b}	38.92 ± 0.07^{c}	76.96 ± 0.58^{c}	54.87 ± 0.44 ^{c}

The values with different superscript letters in a column are significantly different ($p<0.05$).

Serum total cholesterol, s. urea and s. creatinine levels in different groups of Tilipia fish were shown in (Table 5). The results showed that total cholesterol level in G2 was significantly increased as compared with all other groups, where results recorded in G 3 were significantly decreased as compared with other groups. In G4 s. cholesterol was significantly decreased as compared with G2. Cholesterol is a necessary compound of the structure of the cell membrane. It is measured to show the food status in animals. Increased concentrations of cholesterol in serum can bea result of damages to liver or kidney syndrome **(Roohi *et al*., 2015).** Similarly**, (Imanpoor *et al*.,2011)** investigated the effects of chloramine T on common carp and showed that concentration of cholesterol decreased in fish which were exposed to chloramine T. **Metwally, (2009)** recorded that cholesterol levels were significantly decreased in the tilipia fish treated groups with garlic as probiotic.

Moreover, the same pattern of results was recorded for s. urea and s. creatinine in all groups. The results in G2 were significantly increased as comparing with all other groups, where

results recorded in G 3 were significantly decreased as compared with other groups. The results recorded in group 4 were improved toward normal results but significantly decreased compared to results recorded in G2.

Blood urea and serum creatinine are biomarkers for kidney function in fish and more often recommended as an environmental indicator of water pollution than any other organs **(Dos Santos *et al*., 2017)**. In our study we find increases in urea and creatinine concentrations in serum of both groups (2&4) which agreed with histopathological examination

The toxicants and infection cause a disturbance in the physiological state of the fish, which affects the biochemical parameters. It then causes distortions in the cell organelles, which may lead to the elevation in the activity of various enzymes **(Seriani *et al.*, 2011).**

Because the kidney is considered the main excretory organ involved in xenobiotic excretion, its function was investigated in this study, where *P. aeruginosa* led to a significant increase in serum urea and creatinine level .Previous studies reported the same findings with creatinine and urea **(Souza *et al*., 2016 and Dos Santos *et al*., 2017).** Probiotic improve the kidney function and lowered level of serum urea and creatinine and this recorded by many authers **(Metwally,2009)** argued that, the reason of the elevated creatinine levels is due to dysfunction of kidneys.

An investigation into the survival rate of common carp fed Lactobacillus acidophilus and challenged with *P. aeruginosa* and *Aeromonas hydrophila* showed significantly increase survival rates in treated groups than the control which is in agreement with previous **(Araujo, *et al.*, 2015).**

Antioxidant enzymes activity:

The glutathione peroxidase (GPx) activity in serum was highest value recorded in G 3 and lowest one recorded in group 2.

The activity of glutathione-S-transferase (GST) was increased in G 3 as compared with control activity. These values were statistically significantly increased.

Highest level of superoxide dismutase (SOD) in serum were recorded in G3, where lowest values recorded in G2.

Because the blood cells of vertebrates are the primary effect or in host defense involved in various immune processes, the measurement of the oxidative stress status of serum isimportant **(Söderhäll and Cerenius 1998).**

Glutathione-S-transferase (GST) and GPx **(Nugroho and Fotedar 2013)** have been successfully used as tools to evaluate immunity and health status in marron studies.

SOD antioxidant enzymes stimulate removal of peroxides, and superoxide radicals by converts' superoxide anion radical to water and hydrogen peroxide **(Hegazi *et al*., 2015).**

There were significant increase in activity of antioxidant enzymes values seen in group 4 compared to control, this increase in antioxidant enzymes activity in this group due to trying to get rid of free radical which accumulated by *P.aeruginosa* infection.

GPx plays important role in detoxifies hydrogen peroxide in living cells **(Tripathi *et al.*, 2006)**. This reaction plays a very important role in defending the cells and maintaining it from damage which occurring by free radicals, which formed by peroxide decomposition.

The GPx enzymes using glutathione to reduce peroxides into water, therefore preventing the free radicals formation **(Hegazi *et al.*, 2015).**

The antioxidant enzymes play an important role in defend and protect aquatic organisms from free radicals that cause oxidative stress **(Hegazi *et al*., 2010).**

Many studies show that bacterial infection leads to respiratory burst and modifications in antioxidant enzyme activities and their gene expression .Oxidative stress occurs when the rate of production of reactive oxygen species (free radicals) exceeds the scavenging rate by antioxidant molecules **(Adeyemi, 2014).**

Under normal physiological status, the antioxidant defense systems together with SOD, GPx and GST can be affected by way of a slight oxidative stress as a compensatory response.

Thus, the ROS (Reactive Oxygen Species) should be considered to be responsible for protecting the organisms from oxidative damage **(Hindu *et al.*, 2018).**

The recorded decrease in GPx, GST and SOD activity in group 2 while increase enzymes activities in groups 3&4 are agreed with **(Tang *et al.*,2018)** who recorded decrease in SOD and GST activities in fish infected with *A. hydrophila* indicated that the antioxidant defenses of grass carp were weakened, which may cause tissue damages by excessive free radicals.

Zhang *et al*., (2013), stated that, the grass carp fed diets containing *B. subtilis* showed an indication of the enzymatic and non-enzymatic antioxidants activity that neutralize the effects of ROS. The activity of the some antioxidant enzymes in a tilapia fish being superior in the animals injected with PBS when compared to those challenged with *A. hydrophila* **(Liu *et al.*, 2017). Hegazi *et al*., (2010)** who found that, the tilpia fish which exposed to sublethal total

ammonia nitrogen (TAN) showing a negative effect on the activity of GPx and SOD. The activity of these enzymes was increased with increase of (TAN) concentrations.

In Egypt, aquaculture industry, especially Tilapia farming, is growing steadily making a significant contribution to income and food security. Intensive fish farming is associated with a high incidence of stress-related diseases which may need to the use of antibiotics, results in developing antimicrobial resistance that is public health hazards. Probiotics are considered a safe alternative to antibiotics.

Table (6): Organolyptic examination of fresh fish samples.

Goups / Parameters	G1	G2	G3	G4
Eye	**9.40±0.43**	**5.20±0.48**	**9.90±0.32**	**9.30 ±0.18**
Gills	**9.35±1.15**	**5.20±0.63**	**9.60±0.41**	**9.50 ±0.82**
Body surface	**9.50±0.45**	**3.10±0.42**	**9.80±0.61**	**9.65 ±0.47**
Odour	**8.60±0.26**	**5.40±0.41**	**9.90±0.85**	**9 .45±0.48**
Texture	**8.60±0.16**	**3.20±0.31**	**9.80±0.62**	**9.80 ±0.50**
Flesh condition	**8.40±1.45**	**3.30±0.22**	**9.70±0.61**	**9.60 ±0.88**
Visera	**8.50±0.37**	**6.40±0.43**	**9.90±0.41**	**9.80 ±0.37**
Belly wall	**8.40±0.60**	**6.20±0.25**	**9.80±0.65**	**9.60 ±0.62**

The data obtained from sensory evaluation of tilapia fish are presented in (Table 6) which was performed by panelists chosen from the staff members of the Animal Health Research Institute, Tanta Lab. The organolyptic assessment of raw tilapia was made according to the scale described by ten trained **(Barile *et al*., 1985).** This scale ranged from zero (extremely unacceptable) to 10 (highly acceptable) for the following characteristics acceptability, eye pupil, gills, body surface (appearance), odor, texture, flesh condition, viscera and belly wall. The values were calculated as means of ten scoring evaluations. There was a marked reduction in the sensory properties of G2 with great bad changes in the appearance of tilapia fish. Body surface of tilapia was changed to a dull, grey colour slim skin, cloudy eye, dark brown fishy smell gills with sticky mucus materials and soft flesh which found to have attained the border of unacceptability and rejected from consumers, Addition of ABM improve sensory parameters in G3&G4 which have higher overall sensory scores ranged from 9.30 to 9.90 (out of 10) and well accepted to consumers.

Table (7): Effect of addition of ABM on bacterial count of fish meat samples (Values are mean ± S.E.n =3).

Groups	G1	G2	G3	G4
T.B.C	$5 \times 10^4 \pm 1.2 \times 10^{3\,b}$	$2.4\pm10^5\pm7\times10^{3\,a}$	$4\times10^3\pm1.7\times10^{2\,c}$	$4\times10^3 \pm3\times10^{2\,c}$
T. *Staph.aureus*	$2.3\times10^2 \pm3 \times10^{a}$	$2.3\times10 \pm0.88^{b}$	$1.6\times10\pm1.2^{b}$	$1.6\times10\pm1.2^{b}$
T. *enterobacteriacea*	$3 \times10^{2}\pm1.4\times10^{b}$	$4.3\times10^2 \pm4.4\times10^{b}$	0.0 ± 0.0^{c}	$11\times10\pm0.8^{d}$
T. *psychotroph*	$3\times 10^{2}\pm1.4 \times10^{bc}$	$4.7\times10^3\pm2.0\times10^{2a}$	$4\times10 \pm1.7^{c}$	$7.7\times10^2 \pm3\times10^{b}$
p. aeruginosa	0.0 ± 0.0^{c}	$2\times10^3\pm1.0\times10^{2a}$	0.0 ± 0.0^{c}	$2\times10\pm0.21^{b}$
pH	6.11 ± 0.02^{a}	6.53 ± 0.01^{b}	5.91 ± 0.02^{a}	6.01 ± 0.01^{a}

The values with different superscript letters in a row are significantly different ($p<0.05$).

Results obtain from (Table 7) declared that, the mean values pH of fish meat samples was slightly higher in G2(infected) than other groups due to increase in microbial load and volatile bases such as ammonia produced by either microbial or muscular enzymes **(Li *et al*., 2012).** Generally, the natural pH of live fish is just above 7.0, typically about 7.3, but this value falls markedly after death as the fish goes through rigor mortis and glycogen is converted to lactic acid. In most species, the post mortem pH is between 6.0 and 6.8. Differences among the initial pH values may be due to the species, diet, and season, level of stress during the catch as well as type of muscle **(Hernandez *et al*., 2009).**

Probiotics in aquaculture were shown to have several modes of action; competitive exclusion of pathogenic bacteria through the production of inhibitory compounds **(Servin,2004)** improvement of water quality **(Verschuere *et al.,* 2000)**, enhancement of immune response of host species **(Balcáazar *et al*., 2006)** and enhancement of nutrition of host species through the production of supplemental digestive enzymes **(Ziaei-Nejad *et al*., 2006).**So From the achieved data in (Table 7) we realized that G3&G4 had the best bacterial count in comparison with other groups G1& G2 and the reason could be due to the effect of probiotic ABM as anti-bacterial as it retard microbial growth of spoilage and pathogenic bacteria specially with reduction of *P.aeruginosa* from 0.1×10^8 cfu/ml to$2\times10\pm0.21$ in G4.In this respect**, (ICMS,1986)** stated that, the upper acceptability limit of total viable bacterial count in fresh fish is 7 log10 CFU/g flesh, and 6 log10 CFU/g is the maximum permissible limit of TVC recommended by **(EOS, 2005).**

Psychotropic bacteria are very important among different bacteria causing spoilage, because they are mostly related to the changes in sensory attributes such as odour, texture and flavour and could produce different metabolic compounds such as ketones, aldehydes, and biogenic amines **(Safari and Yosefian, 2006).** A proposed limit of psychotropic bacteria is 10^3 to 10^4cfu/g, which is consistent with other studies **(Cascado *et al*., 2005).**

In (Table 7) the psychotropic bacterial count of G2 were increased than other groups, such high counts for this group of bacteria lead to increase in pH and must contribute to the reduction in product shelf life. Increase in the psychotropic bacterial count for the product being sensorially not accepted by the consumer. In contrast the psychotropic count were 4×10 ±1.7 and7.7×10^2 ±3×10 cfu/g respectively in G3&G4 lower than control one were due to the effect of addition of probiotic ABM which improve the quality of fish meat.

The **National Academy of Science (1985)** reported that coliform group of bacteria in fish and fishery products has been considered important in microbiological analysis on account of their significance as indicator organisms for pin pointing the unhygienic conditions during catching, handling, processing and distribution.

There were high significant different in total *enterobacteriacae* count within groups especially in G3 which had a reduction percent 100% in comparison with G1,these results indicated that the uses of ABM had great inhibition effect on total *enterobacteriacae* count and acts as antibacterial with beneficial effects and control of pathogenic bacteria which cause several problem in fish and consumers.

In this study isolation of *Salmonella* , *E coli* and *Aeromonas spp.* were not detected in any of the samples analyzed in the experiment and this may be attributed to that, the water used in the glass aquaria were tap dechlorinated water and there is no sewage pollution and technological characteristics of the fish. Measurement of proximate profiles such as, protein, lipids, TVN and TBA content is often necessary to ensure that they meet the requirements of food regulations and commercial specifications; they also influence postharvest processing and the shelf-life of the fish.

Table (8): Protein and fat % in experimented fish meat samples.

Group No	Fat %	Protein %
G1	1.63±0.2[a]	14.0±0.1[a]
G2	1.61±0.4 [a]	13.1±0.1 [a]
G3	2.64±0.1 [b]	15.9±0.3 [b]
G4	2.13±0.2 [b]	14.7±0.2 [a]

Results are presented as means ± standard error, n = 3.

The values with different superscript letters in a column are significantly different (p<0.05).

Nutritionally, fish is considered an important and rich source of affordable high quality protein and is characterized by desirable composition of amino acids besides its acceptance as balanced source of animal protein and vitamins, fish also provides polyunsaturated fatty acids (PUSFAs) necessary for optimal health.

Table (8) indicated that, the highest protein content was observed in G3 15.9±0.3 g/100 gm compared with control group 14.0±0.1. Similar result were recorded by **(El-Nabarawy, 2017)** who stated that, the total protein content of *O. niloticus* ranged from 12.26 to 16.77 g/100 gm and **(Fanuel *et al.*, 2017**) who recorded that protein content of fresh water fish ranged between 13.86 gm% and 17.12 gm% and they attributed this to the capacity of the fish to absorb and assimilate the essential nutrients from the harvest water - where they habitat-or to the available diet and the amount of dissolved oxygen (DO). Also optimum concentration of DO give the ability of the fish to recover quickly from the physiological stress created by low dissolved oxygen.

In this study high fat %was 2.64±0.1 in G3 then G4 2.13±0.2 and this is agree with **(Gaber, 2000)** who stated that, the normal lipid content of *O. niloticus* is 2.75± 0.16 % .and **(Fanuel *et al.*, 2017**) who recorded fat % as 3.17±0.33, 1.74±0.35 and 1.73± 0.33 from three different ecosystems. There is no difference was noted between G1&G2 in fat content in fish muscle samples. Lipid is used to classify fish **(Ackman, 1989)** as - normally- high-lipid fishes have less water and more protein than low-lipid fishes. These contribute to improving water quality consequently fish health and may be attributed to the enhanced growth of beneficial bacteria in groups treated by ABM and enhancement of nutrition of host species through the production of supplemental digestive enzymes **(Ziaei-Nejad *et al.*,2006).** Using of probiotics and prebiotics has been regarded during recent years as an alternative viable therapy in fish culture, appearing as a promising biological control strategy and becoming an integral part of

aquaculture practices for improving growth and disease resistance **(Rombout *et al*., 2010).** This strategy offers innumerable advantages to overcome the limitation and side effects of antibiotics and other drugs and also leads to high production.

Table (9): Effect of ABM probiotic on TVN, TBA /100 g fish meat samples.

Group No. / Chemical test	G1	G2	G3	G4
TVN	**14.97±0.3 [a]**	**17.64±0.2 [b]**	**13.41±0.42[c]**	**13.13±0.1[c]**
TBA	**0.41±0.02 [a]**	**0.55±0.04 [b]**	**0.37±0.06 [a]**	**0.39±0.07 [a]**

Results are presented as means ± standard error, n = 3.

The values with different superscript letters in a row are significantly different (p<0.05).

TVN value is a quality index for metabolic activity of fish spoilage bacteria and endogenous enzymes action **(Connell, 1990).** A level of 30 mg/100 g has been considered as an upper limit above which fish are considered unfit for human consumption **(EOS: 3494/2005)**. In our study (Table 9), the TVN values were within normal limit in all groups. The average of TVN value in (G1), (G2) & (G3) and (G4) were 14.97±0.3, 17.64±0.2, 13.41±0.42and 13.13±0.1 respectively. High initial value of TVN which observed in G2 could be related to high bacterial count of this group and the growth of microorganisms as proliferation of the microflora contributing to spoilage changes as seen by increased TVN level whereas G3&G4 comparison to G1(control) show low level of TVN due to the effect of probiotic ABM. As a competitive exclusion of pathogenic bacteria. This correlation is in agreement with the findings of **(Balamatsia *et al*., 2007).**

Banks *et al*., (1980) indicated that, the differences in TVB-N amounts must have been caused by a smaller number of bacteria their ability to act on the oxidative desamination of non-protein nitrogen compounds and showed a clear relationship between the microbiological quality of meat (protein based) and the total amount of Total Volatile Nitrogen (TVN) and biogenic amines.

TBA is a significant quality index for fatty fish **(Lynch and Frci, 1993)**.The TBA factor is responsible for a rancid flavor, off odor, color as well as texture deterioration **(Olafsdottir *et al*., 1997).** According to **EOS, (2005)** the permissible limit of TBA was 4.5 mg MDA/kg. In our study (Table 9), the TBA values were within normal limit in (G1), (G3) and (G4),

The average were 0.41 ±0.02, 0.37±0.06 and 0.39±0.07respectively, while high value of TVN was observed in G2, G3&G4 comparison to G1(control) show low level of TBA due to the effect of probiotic ABM.

Table (10): Reisolation of *P.aeruginosa* from infected fish.

Time of collection	Group No.	No. of examined fish	Positive samples of *P. aeruginosa*			Total	
			Muscle	Liver	Gills		
			No	No	No	No	%
Week4	G1	3	0	0	0	0	0
	G2	3	3	3	3	9	100
	G3	3	0	0	0	0	0
	G4	3	0	0	0	0	0
Week5	G1	3	0	0	0	0	0
	G2	3	3	3	3	9	100
	G3	3	0	0	0	0	0
	G4	3	0	2	2	4	44.4
Week6	G1	3	0	0	0	0	0
	G2	3	3	3	3	9	100
	G3	3	0	0	0	0	0
	G4	3	0	1	1	2	22.2
Week7	G1	3	0	0	0	0	0
	G2	3	3	3	3	9	100
	G3	3	0	0	0	0	0
	G4	3	0	1	1	2	22.2
Week8	G1	3	0	0	0	0	0
	G2	3	3	3	3	9	100
	G3	3	0	0	0	0	0
	G4	3	0	1	1	2	22.2

***The percentage was calculated according to the total number of each group organs (n = 9).**

G1: group feed basic diet only.

G2: group infected with *P. aeruginosa* without treatment.

G3: group fed on probiotic from beginning to the end of experiment.

G4: group treated with probiotic and infected with *P.aeruginosa* at the 21th day of the experiment.

Table (10) explain reisolation of *P.aeruginosa* in G2 (positive control), the incidence of *P. aeruginosa* from muscle, liver and gills were 100% at fourth, fifth, sixth, seventh and eighth week of experiment. while the incidence of reisolation of *P. aeruginosa* in G4 (prophylactic with ABM probiotic from beginning to the end of the experiment) from muscles ,liver, gills were 0%, 44.4%, 22.2%, 22.2% , 22.2% respectively at fourth week, fifth ,

sixth, seventh, and eighth respectively. These may be attributed to probiotic (ABM) which caused alteration in the microbial metabolism, stimulation of the immune system of Tilapia fish have positively impacted resistance of fish.This result agrees with**(Gatesoupe*et al.*, 1999).**

Molecular identification of *P. aeruginosa* 16 SrDNA, ExoS, lasB, toxA. Genes used for confirmation of *P. aeruginosa* are explain in Fig. (A).

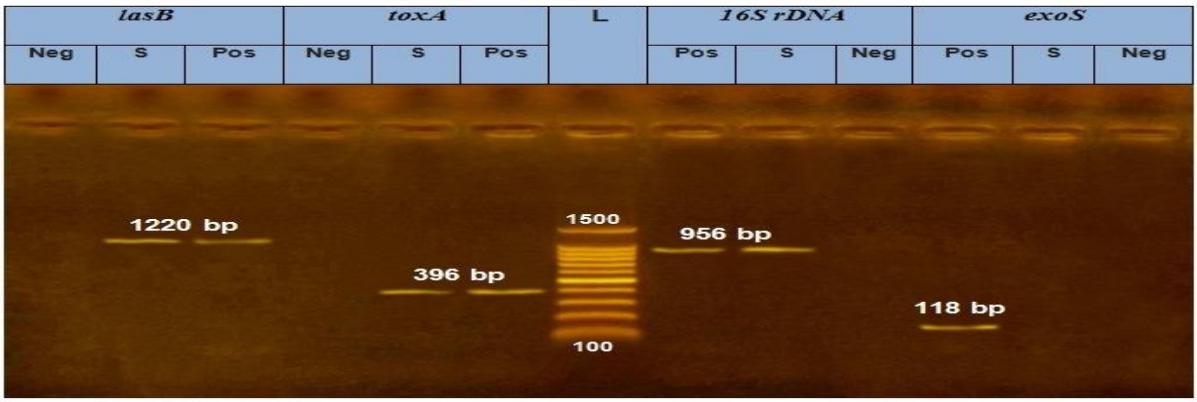


Fig. (A): Agarose gel electrophoresis showing amplification of 1220 bp, 396 bp , 956 bp and 118 bp fragment using *las*B, *tox*A ,6SrDNA primers and *exo*S M) respectively, 100 bp DNA Ladder, control positive, control negative.

Neg: Negative control.

Pos: Postive control.

L: DNA ladder (marker) 100-1500.

LasB positive for sample at 1220 bp.

ToxA positive for sample at 396 bp.

16SrDNA positive for sample at 956 bp.

ExoS negative for sample.

Fig. (A) showed virulence gene (LasB, ToxA) in challenged bacteria which is responsible for pathogenic effect of *P. aeruginosa*. *P.aeruginosa* produces several virulent factors to colonize the cells of its host. Many of these factors are controlled by regulatory systems involving cell-to-cell signaling **(Van Delden and Iglewski,1998).** Among these are*:* exotoxin A, Las B elastase. Exotoxin A, encoded by the *tox* A gene, inhibits protein biosynthesis by transferring an ADP- ribosyl moiety to elongation factor 2 of eukaryotic cells. As B elastase, a zinc metalloprotease encoded by the las B gene, attacks eukaryotic proteins such as collagen and elastin, and destroys the structural proteins of the cell **(Toder *et al*., 1994).**

P. aeruginosa was identified in various species of fish as causative agents of *Pseudomonas septicemia***(EL-Nagar, 2010),** which characterized by fin rot , petechial hemorrhage, darkness of the skin, detached scales, abdominal ascitis and exophthalmia **(Khalil *et al*., 2010).** *Pseudomonas septicemia* is one of the important pathogenic bacteria affecting fish farm in Egypt **(Khalil *et al*., 2010).**

In Egypt, aquaculture industry, especially tilapia farming, is growing steadily making a significant contribution to income and food security. Intensive fish farming is associated with a high incidence of stress-related diseases which may need to the use of antibiotics leads to result in developing antimicrobial resistance that is public health hazards. Probiotics are considered a safe alternative to antibiotics.

This study was done to assess the ABM probiotic as antibacterial against *P.aeruginosa* infection and disease resistant, investigate its effect as a dietary supplementation, growth promotor, water quality of cultured Tilapia fish

6. Immunological Assay:

Effect of ABM probiotic supplementation to Tilapia fish experimentally infected with *P. aeruginosa* on some immunological parameters of fish are shown in (Table 11)

Results of lysozyme activity in serum samples revealed significant elevation in group inoculated with *P.aeruginosa* (G2) compared to control group (G1). On the other hand, administration of ABM probiotic to group challenged with *P. aeruginosa* modulated the increment in lysozyme activity significantly. Meanwhile, lysozyme serum activity of ABM probiotic administered group (G4) showed significant increase compared to control negative group. Lysozyme activity constitutes an essential defense mechanism against pathogens in fish, and the bacteriolytic activity of lysozyme in fish skin mucus and other tissues contributes to the host defense mechanism against bacterial infection **(Sanchooli *et al.*, 2012).** Meanwhile, lysosomes are multifunction cellular organelles that have important role in intracellular digestion in phagocytic cells due to its enzymatic contents. Lysozymes was known to be one of lysosomal enzymes attacks mucopeptide in cell walls of various bacteria **(Moore *et al.*, 2006)** and have bactericidal activity during phagocytosis **(Tizard,1996).** Therefore, the increase in lysozyme may be due to its leakage from lysosomal granules. Since the toxicant binds to metallthionin and the complex formed is stored in lysosomal granules when the storage exceeding their storage capacity may be bursting of lysosomes and release of lysosomal granules **(Bussolaro *et al.*, 2008).** Our results are in agreement with that of

(Magda *et al.*, 2011) who investigated the effect of dietary supplementation of the probiotics (*Bacillus subtilis*, *Lactobacillus plantarum*, a mixture of bacterial isolates, *B. subtilis*and *L. plantarum*) and the yeast *Saccharomyces cerevisiae* on the immune response of the Niletilapia, Oreochromis niloticus. Experimental results showed significantly increased lysozyme and total immunoglobulin activities in blood samples of the fish supplemented with ABM probiotic compared to those on the control diet. Obviously, the effects of probiotics on immune response and bacterial loading in aquatic organisms and the environment are well documented. The administration of probiotics by live food and/or culture water dramatically decreased bacterial activity in some teleosts **(Suzer *et al.*,2008).**Furthermore, serum lysozyme activities, and serum immunoglobulins level of *E. coioides* were high in Bacillus-treated fish groups **(Sun *et al.*, 2010).**

In the present study, the significant increase in lysozyme activity of fish fed different probiotics suggests an immune stimulation. Lysozyme is an enzyme with antibacterial activity that can split peptidoglycan in bacterial cell walls particularly the gram positive species and it can cause lysis of the cells **(Chipman and Sharon, 1969).** Lysozyme occurs predominantly in fish mucus, serum and tissues rich in leucocytes **(Ellis, 1999).** Infections or invasion by foreign material could result in an increase in the lysozyme concentration in fish blood **(Moyner *et al*., 1993).**

Serum nitric oxide (NO) assay revealed significant elevation in group inoculated with *P. aeruginosa* compared to control negative group. Improvement of inoculated fish occurred when co-administration of ABM to *P. aeruginosa* challenged group (G4) significantly as compared with group inoculated with *P. aeruginosa* alone (G2). On the other hand, fish supplemented with ABM probiotic stimulated NO production significantly compared to control. Nitric oxide is bactericidal reactive oxygen that is produced initially by macrophages following stimulation with a variety of agents, as microbial component and cytokines **(Gomez-Gil *et al.*, 2000).**

Nitric oxide (NO) is physiologically catalyzed by NO synthetase which has important and several roles in immune response as a toxic agent towards infectious agents, an inducer or suppressor of apoptosis or as immune modulator **(Colman,2001).**This findings of mounted immune response in the challenged and treated fish may be due to the higher concentration of NO which can be rapidly converted to other reactive nitrogen oxide intermediates that could

be quickly damage different fish liable cells in blood, anterior kidney, spleen and other parts of the fish immune system. This could be frankly and practically declared the higher level of NO in *P. aeruginosa* infected group **(Alderton *et al.*, 2001)**.

Our results are in agreement with **(Selim and Reda, 2015)** who recorded that serum (NO) was increased by the addition of amyloliquefaciens at levels of 10^4 and 10^6 CFU/g to the tilapia diet and declared that it improves NO production in In- dian carp when given at different doses of diet . Dietary supplementations with Bacillus subtilis induced significantly higher NO levels in sea cucumbers **(Zhao *et al.*, 2012)**.

Values of total serum protein in group intoxicated with *P. aeruginosa* decreased significantly compared to control. However, treated fish group with ABM probiotic modulated the hypoproteinemia significantly. Moreover, ABM probiotic treated group (G4) behaved rather similar to control during the experimental period.

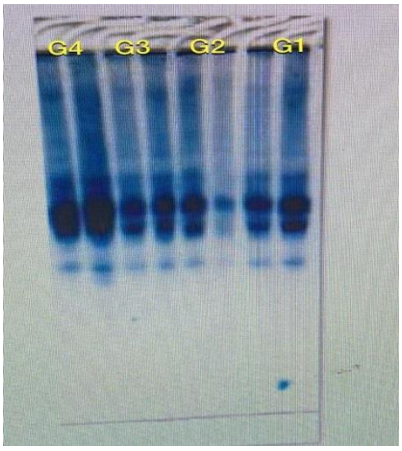


Fig. (B): Electrophoretic pattern of serum protein of Tilapia fish administered and\or entoxicated with *P. aeruginosa.*

According of electrophoretic pattern of serum protein pointed out the presence of 9 fractions; *P. aeruginosa* infected group (G2) provoked a significant decrease in both total protein, albumin may represent fraction 1&2 and globulins may represent fractions 3-9 compared to control group (G1). On the other hand, these observations encountered by pretreatment with ABM probiotic to fish intoxicated with *P. aeruginosa* (G4) which showed elevation in total protein, albumin and globulins compared to control +ve (G2) group. Whereas, albumin and globulin fractions revealed significant increase in group administered ABM probiotic alone (G3) compared to control -ve group (G1).

Table (11): Effect of ABM on some immunological parameters of Tilapia fish.

	Lysozyme	N.O.	T.P.	Albumin	Immunoglobulin
G1	**461.90±42.27**	**4.41±0.21**	**4.79±0.29**	**2.03±04**	**2.75±0.47**
G2	**718.97±132.9***	**8.43±0.52***	**3.47±0.41***	**1.14±0.15***	**2.33±0.48**
G3	**630.64±87.07***	**5.68±0.49***	**5.35±0.45***	**1.77±0.69**	**3.58±0.65***
G4	**492.38±113.54***	**5.65±1.12***	**4.87±0.12**	**1.30±0.23***	**3.57±0.23***

*** is significant at p ≤ 0.05 Different groups compared to control Values represent means ± SD**

These results are agreed with those of **(Adonova *et al*. 2014)** who recorded hypoalbuminemia in dogs infected with *P. aeuroginosa*. They attributed the decrease in albumin level to increased vascular permeability due to bacterial toxins resulting in the passage of albumin to the surrounding interstitial tissue. Also, with those of **(Ramalingam and Ramarani, 2006).** In this respect, **Saad *et al.*, (2014)** discussed the decrease in protein and globulin that can explain the drastic effect of *Pseudomonas* infection on immune response of infected fish with subsequently increased the drastic damage effects of bacterial diseases. The causes of decreased serum total protein may be attributed to decrease of albumin concentration which occurred after vascular leaking due to increasing permeability after histamine release **(Ellis,1981)**, liver damage and anorexia, non - specific proteolysis.

The total immunoglobulin results showed significantly higher values in fish supplemented by ABM probiotic as compared to the control. This finding confirms that, the probiotic bacteria can stimulate the antibody production in fish **(Panigrahi *et al*.,2004).**Serum immunoglobulins are major components of the humoral immune system which provide disease protection in animals and humans **(Watts *et al*., 2001).**Furthermore,**(Panigrahi *et al*., 2004)** reported an increase in total serum immunoglobulin levels of rainbow trout, Oncorhynchus mykiss and the Indian major carp, *Labeo rohita*, fed diet containing *B. subtilis* **(Nayak *et al*., 2007).**

In addition, Yeast cells have been found tobe effective e in increasing the serum immunoglobulin IgM level of seabream, Sparus aurataby its supplementation to the diet as an immune stimulation **(Cuesta *et al*., 2004).**

In the present study, an improvement in several immunological parameters were elaborated mainly due to the administration of ABM to the diet. The findings of our study suggest a possible synergistic action between components of ABM probiotic (*Lactobacillu*s spp,

Bacillus subtilis and *Bacillus licheniforms*. Yeast extract *(Saccharomyces cerevisiae),* 5 Safe plant extract, dipotassium hydrophosphate, calcium Carbonate and natural colours) that gave the highest values for lysozyme activity and total immunoglobulin in fish fed ABM. However, the combination of bacterial strains occupy different niches within the gut microflora environment may in turn have desirable effects on the host immune response and health of gilthead sea bream and O. niloticus **(Aly *et al.*, 2008)**. So, viability of probiotics as a factor influencing the immune response in the Tilapia fish.

7. Pathological Results:

The results showing that fish with infection with *P. aeruginosa* revealed different pathological changes in gills, skin, muscles, liver, kidneys and gonads. Gills showed necrosis and destruction of epithelial cells, fusion of gills lamellae associated with accumulation of inflammatory cells Fig. (1), while protected and infected fish (G4) revealed improvement of gills structure and gill lamellae retain to normal radiating structure Fig. (2) while the fish treated only with probiotics (G3) revealed dilation of blood capillaries and increase blood supply to the gills lamellae with proliferation of chloride cells Fig. (3) the treated groups with *P. aeruginosa* and probiotics showed improvement of epithelial structure of gills and lower inflammatory cells in compared with the control group which revealed accumulation of inflammatory cells in the epithelium of gills in addition to proliferation of theses epithelial cells resulting in fusion of gills lamellae. That results were in agreement with **(Wael *et al.*, 2009)** who stated that congestion in the gill arch was seen and mononuclear cells were infiltrated the top and base of the primary lamellaein fish treated with probiotic. The skin in infected groups by *P. aeruginosa* revealed ulceration of the epidermal cell layers with necrosis of the epidermal cells Fig. (4) Increase rod let and mucus cells in the epidermal cells layers Fig. (5), while in the group administered ABM then infected with *Pseudomonas* (G4) showed degeneration of the epidermal cells layers and proliferation of club cells Fig. (6). The control group showed normal skin structure with normal club cells as group treated with probiotics and *Pseudomonas* and these results supported by **(Pinoargote and Ravi Shankar 2018),** who concluded that probiotic microbes have the potential to secrete inhibitory substances to bacteria and have beneficial effects in improving the water quality of aquaculture systems these ability of probiotic help improving the health of the skin by degrading organic matter and converting it intoco2.The muscles in infected fish with *P. aeuroginosas*howed severe necrotic muscular system with sever loss of muscle striation

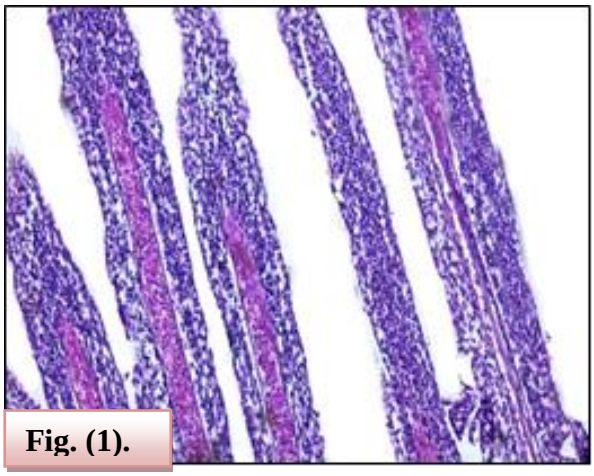
and necrotic destructed sarcoplasm Fig.(7) however in groups infected and treated by probiotics revealed marked improvement of muscular system and normal muscle striated sarcoplasm appeared with prominent muscular fibers nuclei Fig. (8), the groups treated only with ABM probiotics showed heavy good musculature with high muscular striation Fig.(9) the control ones showed normal musculature but less than groups treated with probiotics only and nearly similar to group treated with *Pseudomonas* and probiotics and that results supported by **(Kalaimani and Parvathi, 2017)** who found that the muscle in fish treated with probiotics revealed the bundles of skeletal muscle fibers with striated appearance and marked heterotrophy with increased in cytoplasmic size and increased cell proliferation and cytoplasmic size indicated the better growth. **(Allameh *et al*. 2017)** who stated that probiotics enhance the growth performance .The liver of infected group (G2) showed severe necrotic hepatocytes with loss of nuclei in the hepatopancreas also infected groups showed necrosis of pancreatic acini and loss of their nuclei Fig. (10) while in protected and infected (G4) revealed improvement of hepatocytes with prominent hepatic nuclei and improved hepatopancreatic acini Fig. (11). in groups treated with probiotics only showed sinusoidal congestion filled with blood, hepatocytes with prominent basophilic nucleit nuclei, increase lipid infiltration Fig.(12) high yield proliferation of pancreatic granules secretions with activated dividing nuclei of proliferated acini and healthy hepatocytes with prominent basophilic nuclei Fig.(13). G1& G3 showed normal hepatocytes structure and nearly similar to group treated with Pseudomonas and probiotics but differ from group treated with probiotics only as no congestion in liver sinusoids. **Pinoargote and Ravishankar (2018)** concluded that Probiotics provide an effective approach to reduce acute hepatopancreatic necrosis in shrimp ponds and added that probiotics have inhibitory effects against the Vibrio parahaemolyticus strain that causes acute hepatopancreatic necrosis.

The results in kidneys showed the kidneys of G1& G3 treated with probiotics showed increase vaccuolations of renal tubules and dividing nuclei of renal tubules Fig.(14) severe dilation of the glomerular capillaries and increase active dividing nuclei (In normal manner) with basophilic cytoplasm. The G2 kidneys showed dilatation of glomerular capillaries and necrosis of renal tubular epithelial lining. Renal tubules in kidneys of fish in G4 infected with *Pseudomonas* and treated with probiotics showed proliferation of heamatopiotic tissues Fig. (15) G1 &G3 showed normal glomerular structure and normal renal tubules in compared

with group treated with *Pseudomonas* and probiotics that agreed with **(Wael *et al.*, 2009)** who found minimal pathological changes in kidneys in fish treated with probiotics mainly vacuolar degeneration of some renal tubular epithelium. Focal hyperplasia in the hematopoietic tissue was evident. **Sharma *et al.*, (2013)** stated that fish given the treatment of probiotic showed maximal value of erythrocyte count and increase in the level of different hematological parameters.

Allameh *et al.*, (2017) considered probiotics as an alternative strategy have been suggested to be used as replacement for antimicrobial drugs and growth promoters. In addition, they believed that probiotics have advantages for improving the health of fish in aquaculture and increasing fish performance. The gonads of fish male in infected group with *Pseudomonas* (G2) showed atrophied in the germinal cells layers in semineferous tubules Fig. (16**)** while in group infected and treated with probiotics (G4) showed regeneration of germ cells Fig. (17). in fish females infected groups showed ovarian follicles depletion of oogonia quantity Fig. (18) while in fish infected with *Pseudomonas* and treated with probiotics (G4) showed filling of follicles with oogonia Fig. (19) and that results were in agreement with **(Rahman *et al*,.2018**) who stated that female probiotics enrich dietary effect on the reproductive performance growth. *P. aerugenosa* can be considered as accountable fish infection under culture condition. The use of ABM for 2 month could increase the survival rate and the resistance of fish to some infection diseases. In conclusion, common tilapia fed with diets fish and other fortified ABM increased the non-specific immune parameters. Also protect fish against pathogenic *P.aeruginosa*. Therefore, diets fortified ABM is recommended to improve the non-specific immune response of fish and elevate its protection against pathogenic bacteria. Also, application of probiotic, ABM, in fish water aquaria can be used as antioxidant so improve the growth performance in fishes under stress, increase in protein and fat ratio in muscle, quality of fish water aquaria and quality tests of fish meat samples. This may be attributed to enhancing the immune response and act as a prophylactic effects against a challenge infection and reduce tissue damage it was concluded that probiotic,ABM,can be used in control and prevention of *P .aeruginosa* infection, improving fish growth and meat quality of fish meat samples.

This study was done to assess probiotic ABM as antibacterial against *P. aeruginosa* infection and disease resistant, investigate its effect as a dietary supplementation, growth promoter, water quality and meat quality of Tilapia fish.



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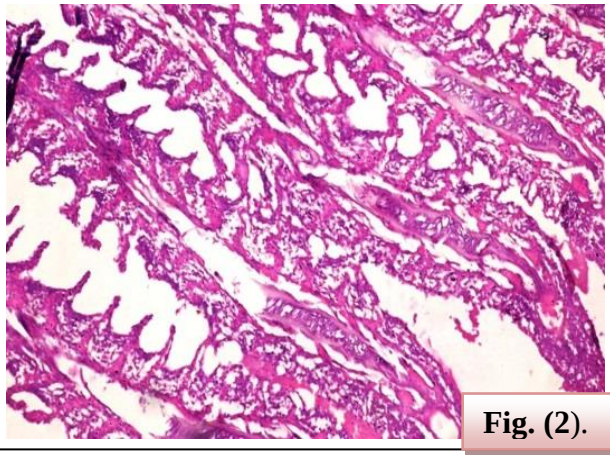

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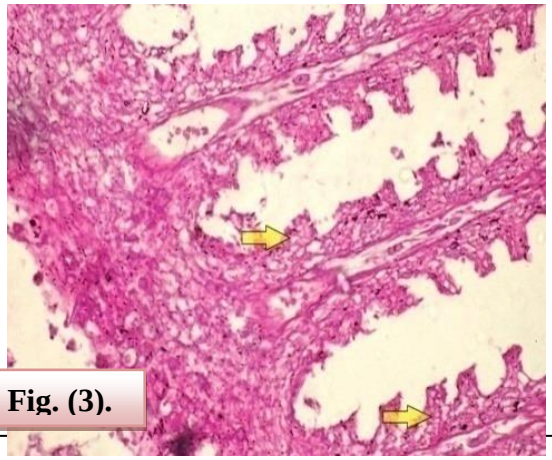

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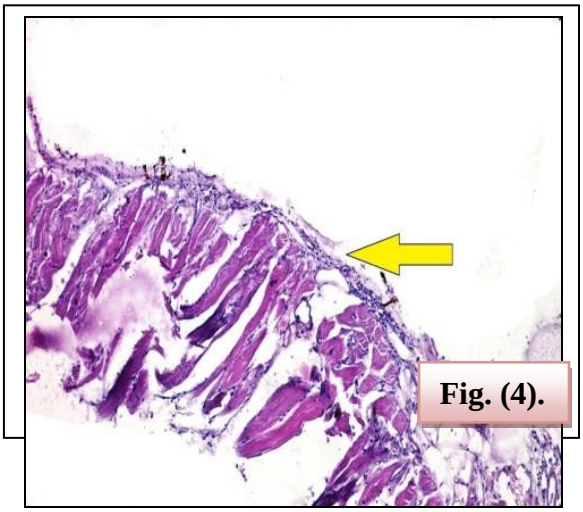

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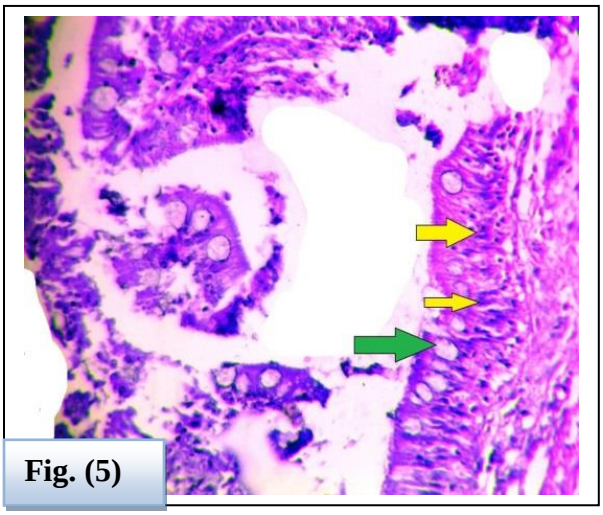

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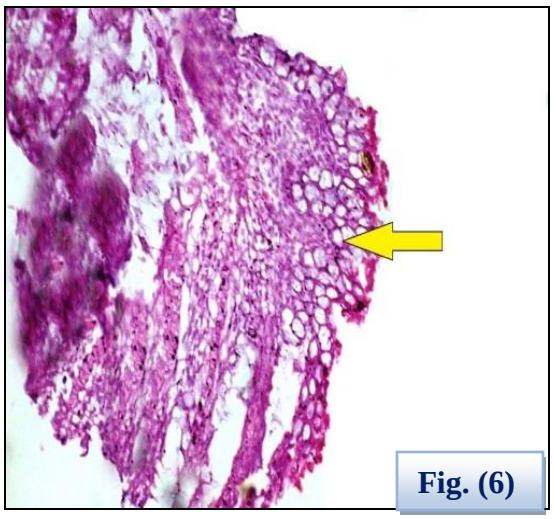

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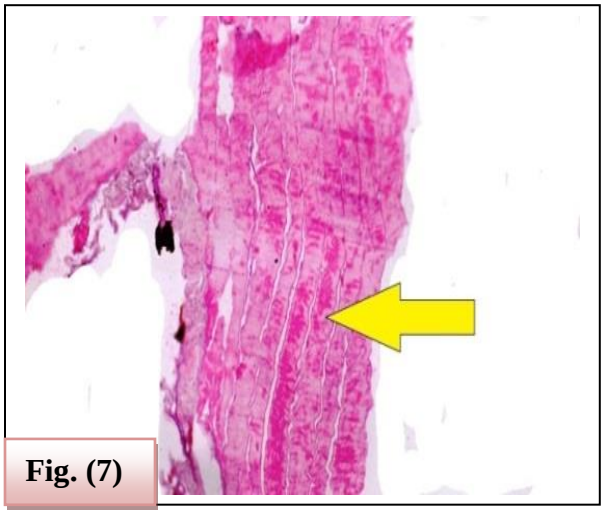

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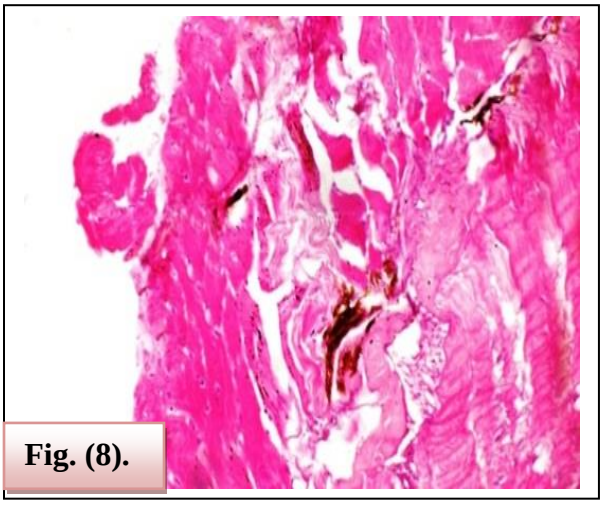

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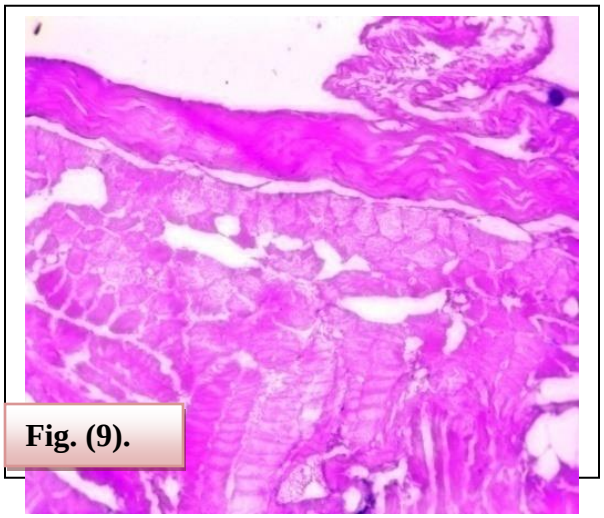

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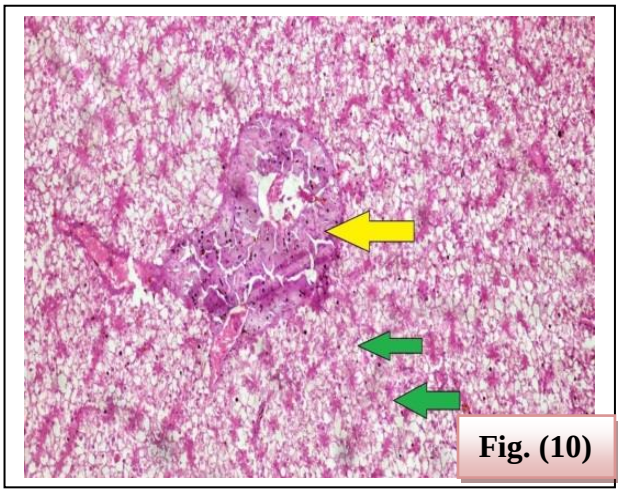

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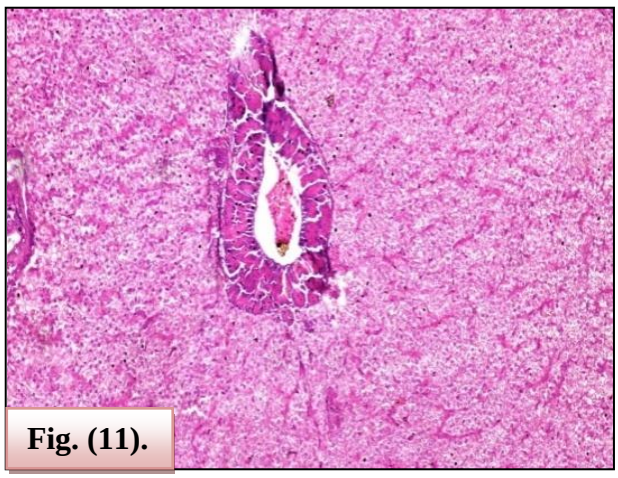

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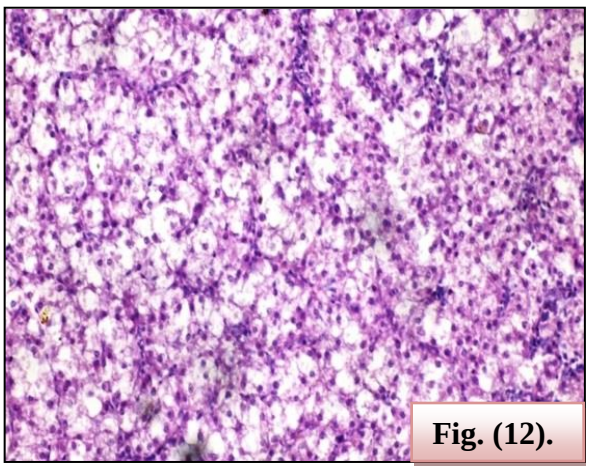

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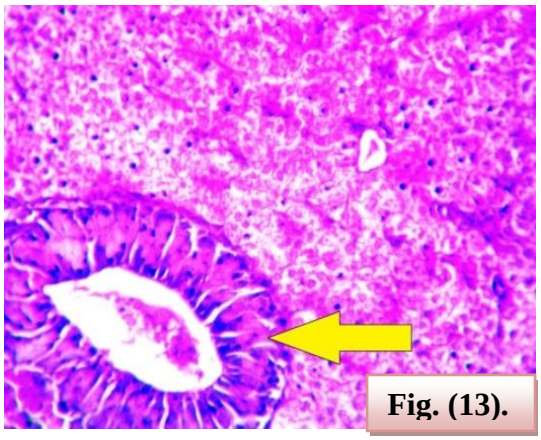

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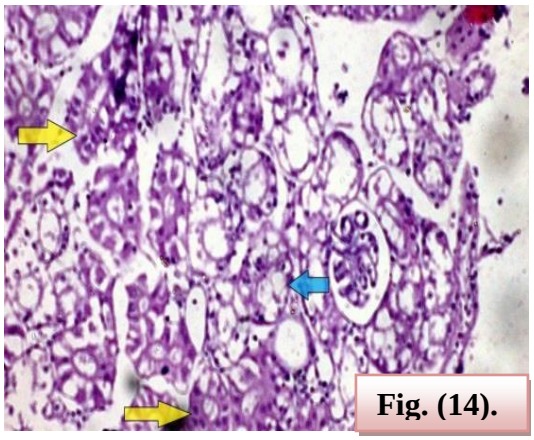

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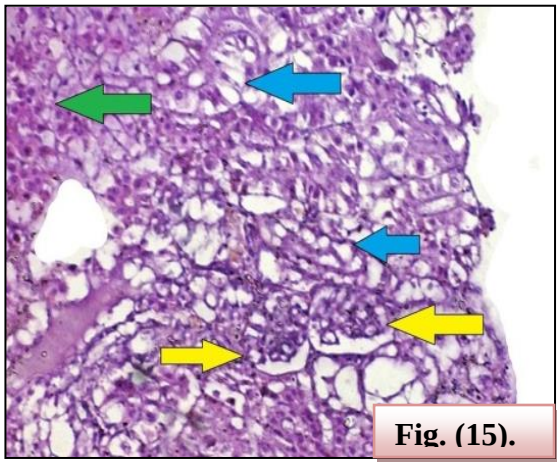

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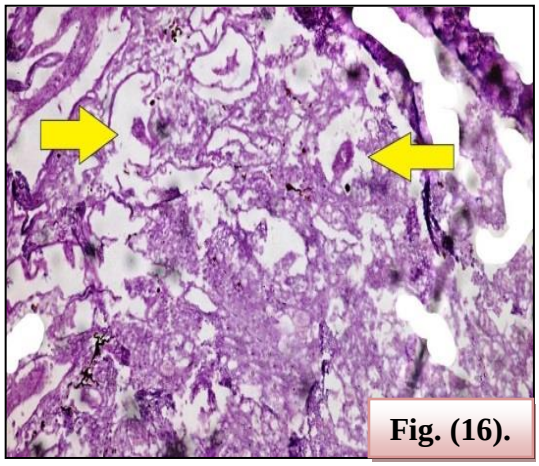

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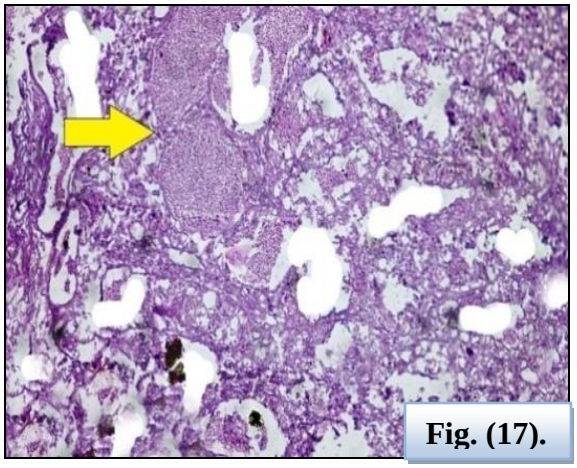

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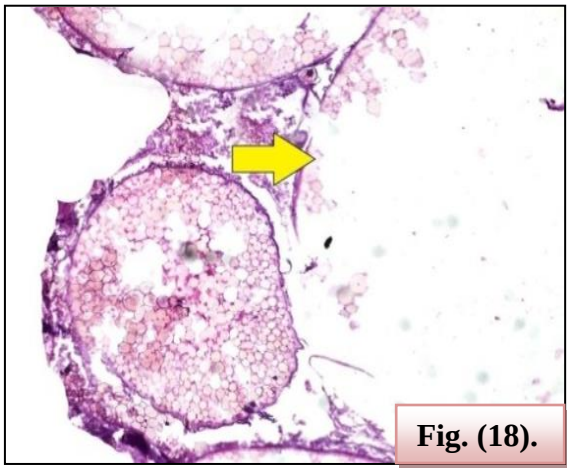

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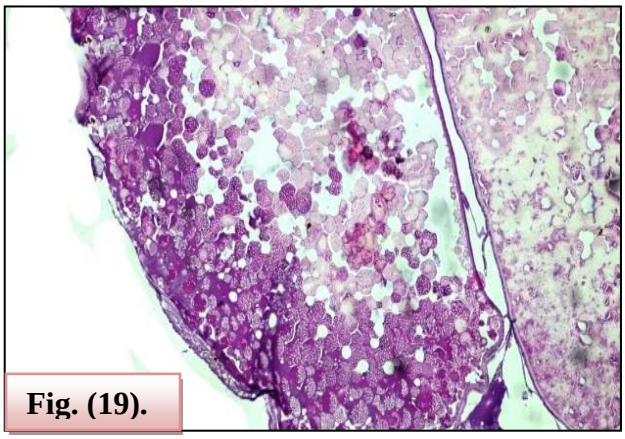

Fig. (19).

Fig.(1): group of fish infected with *P.aeruginosa* fish gills showing fusion of gills lamellae associated with accumulation of inflammatory, associated with necrosis of the epithelial cells of gills lamellae **(H&E , 200x).**

Fig.(2): group of fish treated with probiotics and infected with *P.aeruginosa* showing improvement of gill structure (gill epithelium) and retain to normal radiating structure with absence of inflammatory cells **(H&E, 200x).**

Fig. (3): group of fish treated with probiotics only showing increase blood supply in the blood capillaries of gills and proliferation of chloride cells **(H&E, 200x).**

Fig.(4): group of fish infected with *P.aeruginosa* skin showing complete loss of epidermal cells layers,(yellow arrow) ulceration of epidermal cells layers **(H&E, 100x).**

Fig. (5): group of fish infected with *P.aeruginosa* other part of skin showing Increase rodlet cells (yellow arrows) in the epidermal cells layers and mucuscells (green arrow) **(H&E, 400x).**

Fig. (6): group of fish infected and treated with probiotics showing regeneration of the epidermal cells layers and proliferation of club cells (yellow arrow) **(H&E, 200x).**

Fig. (7): Group of fish infected with *Pseudomonas* showing severe necrosis of muscle structure and loss of muscle striation (necrotic destructed sarcoplasm **(H &E, 200x).**

Fig.(8): group of fish infected with *Pseudomonas* and treated with probiotics showing marked improvement of musculature and the normal muscle striated sarcoplasm appeared with prominent muscular fibers nuclei **(H&E, 200x).**

Fig. (9): group treated with probiotics showing heavy good musculature with high muscular striation **(H&E, 200x).**

Fig. (10): Liver of group infected with *Pseudomonas* showing severe necrotic hepatocytes with loss of nuclei (green arrows) ,necrosis of hepatopancreatic tissues and less pancreatic granules secretions (yellow arrow) with loss of nuclei of pancreatic acini **(H&E, 100x).**

Fig. (11): Liver of group treated with *Pseudomonas* and probiotics showing improvement of hepatocytes structure with good prominent nuclei, the hepatopancreatic tissues showing improvement of acini and increase pancreatic granule **(H &E, 200x).**

Fig.(12):Liver fish with probiotics only showing sinusoidal congestion filled with blood,hepatocytes with prominent basophilic nuclei ,increase lipid infiltration **(H &E , 400x).**

Fig. (13) : Liver of group treated with probiotics showing high yield of pancreatic granules secretion (yellow arrow (with activated dividing nuclei of proliferated acini and healthy hepatocytes with prominent basophilic nuclei **(H&E,400x)**

Fig.(14): The kidneys of groups treated with probiotics showed increase vaccuolations of renal tubules (blue arrow) and dividing nuclei of renal tubules (yellow arrows) **(H&E ,400x)**

Fig. (15): kidneys of fish groups infected with *Pseudomonas* and treated with probiotics showing dilatation of glomerular capillaries (yellow arrows) and proliferation of heamatopiotic tissues green arrow vaccuolations of renal tubular epithelium**(H&E, 200x).**

Fig.(16) :group of fish male gonads showing atrophied in the germinal cells layers in semineferous tubules **(H&E, 200x).**

Fig. (17): group of fish male treated with probiotics showing regeneration of the germinal cells layers in the seminiferous tubules **(H&E, 200x).**

Fig. (18): Gonads fish female in infected groups showing ovarian follicles (yellow arrow) devoid of oogonia **(H &E, 200 x).**

Fig.(19):gonads female treated with probiotics showing filling of follicles with oogonia**(H&E,200 x).**

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تاثير اضافه مستحضر محسنات الغذاء علي التقييم البكتريولوجي والكيميائي وجودة اللحوم و النمو والباثولوجي في اسماك البلطي المعامله بميكروب السودوموناس ايرجنوزا

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مركز البحوث الزراعي

الملخص العربى

تهدف هذه الدراسه لتقييم تاثير المخصب الحيوى البروبيوتك (ABM) علي الناحيه البكتريولوجيه والمناعيه وكذلك نمو السمك وجوده اللحوم على سمك البلطى المعدى بميكروب سودوموناس ايرجنوزا بالاضافة لاجراء الاختبارات الكيميائية والهستوباثولوجية الخاصة بهذة الأسماك وأيضا الوقوف على مدى تأثير (ABM) على كفاءة أحواض المياة ونسبة الأمونيا والأكسوجين الذائب كأحد المشاكل الرئيسية التى تواجة الأستزراع السمكى.

اجريت الدراسه لمده شهرين علي 120 سمكه (15-20 جم) قسمت بالتساوي علي اربع مجاميع. المجموعه الاولي مجموعه ضابطه والمجموعه الثانيه تم عدواها بميكروب سودوموناس ايرجنوزا عن طريق الغشاء البريتوني بتركيز1,.×10^8خليه/ميلليلتر لكل سمكه. المجموعه الثالثه اعطيت المحسن الغذائي (ABM) بجرعه 1لتر /10 م^3 اسبوعيا من بدايه التجربه حتي نهايتها والمجموعه الرابعه اعطيت محسن غذائى (ABM) 1لتر/ 10م^3 اسبوعيا من بدايه التجربه حتى النهايه وتم عدواها بميكروب سودوموناس ايرجنوزا.

. وقد تم قياس الاكسجين و الامونيا يوميا و وزن السمكه اسبوعيا واظهرت النتائج انخفاض نسبه الامونيا فى ماء احواض السمك لكل من المجموعه 3 و 4 عن المجموعه الضابطه ايضا زياده نسب الاكسجين فى ماء احواض السمك لكل من المجموعه 3 و 4 عن المجموعه الضابطه كما لوحظ استمرار مياة الأحواض للمجموعة 3 مع تغيير جزئى لمدة 42يوم كاملة من بداية التجربة لملاءمة نسبة الأمونيا والأكسجين بالاضافة الى زياده ملحوظه فى وزن السمكه فى المجموعه 3 و 4 المضاف لها المحسن الغذائى مقارنه بالمجموعه الضابطه و المعديه 1 و2 على التوالى .

يتم عزل السيدومونس أريجونوزا كل اسبوعين حتى نهاية التجربه وعمل ال PCR ولوحظ اختزال ملحوظ لعزل الميكروب فى المجوعة 4 وقد اوضحت النتائج ان اضافه المحسن الغذائى اختزلت نسبه العد الميكروبي للسودوموناس ارجنوزا مع امكانية استخدامة كعلاج ووقاية لعدوي السودوموناسايرجنوزا.

كما أوضحت النتائج ان هناك فرق معنوى واضح فى العد الكلى البكتيرى و العد الكلى للبكتريا المعويه و العد الكلى للبكتريا المحبه للبروده و العد الكلى للمكروب العنقودى الذهبى بين كل من المجموعه الضابطه و المجموعه الثانية التى تم عدواها وبين المجموعتين 3 و 4 نتيجة اضافة المحسن الغذائى بينما يعزى عدم عزل ميكروبى التسمم الغذائى السالمونيلا

والاشريشيا كولاى لاستخدام مياة الصنبور الخالية من التلوث . أيضا أوضحت النتائج زيادة معنوية فى نسبة البروتين والدهون للمجموعة 3 بالمقارنة بالمجاميع الاخرى بينما لم يوجد تغير واضح لنسبة الدهون بين المجموعتين 1و2. وأظهرت المجموعة الثانية زيادة معنوية فى نسبة TVN, TBA والذى يعزى الى زيادة العد الميكروبى لهذة المجموعة وظهور علامات الفساد مقارنة بانخفاض ملحوظ فى المجموعات 3و4 نتيجة اضافة المحسن الغذائى.

وبالنظر الي الفحوص الكيميائيه لمصل الدم (الدهون في الدم ونسبه الكرياتين ونسبه اليوريا في الدم) وجد ان هناك تحسن وانخفاض ملحوظ لهذه النسب في مصل دم الاسماك التي تمت معالجتها بالمستحضر بعد مقارنه النسب التي سجلت في مجموعه 3 و4بمقارنتها بنسب المجموعه الضابطه. اما بالنسبه لقياس نشاط الانزيمات المانعه للتاكسد وجود ان هناك تحسن وزياده في نسب الانزيمات التي تم قياسها (GXP,GST,SOD) في المحموعات التي تم امدادها وعلاجها بالمستحضر .

كما أوضحت النتائج زيادة معنوية لنشاط اللايسوزيم واكسيد النيتريك في سيرم مجموعة الأسماك التي تم إضافة المحسن الغذائي اليها مقارنة بالمجموعة الضابطة.وبفحص الاميونوجلوبيولين بواسطة التحليل الكهربائي وجد زيادة معنوية بها في المجموعة التي تم تغذيتها بالمحسن الغذائي وذلك مع تحسن معنوي ملحوظ في بروتينات وزلال الدم في المجموعة التي تمت حقنها بميكروب السودوموناسايروجينوزا واضافة المحسن الغذائي.وهذا يوضح أهمية إضافة المحسن الغذائي لرفع مناعة الأسماك وتحفيز الإستجابة المناعية لمقاومة العدوي المختلفة للحفاظ علي حياته وانتاجيته.

كما اظهرت الدراسة الهستوباثولوجية لعينات من الخياشيم والجلد والعضلات والكبدوالكلي والمناسل تجمع للخلاياالالتهابية في الخياشيم و التحامها. تنكرز في كل من الطبقات الجلدية و الكتلة العضلية وخلايا الكبد و كذلك خلايا الكبد البنكرياسية. وجود تكاثر في النسيج الدموي الكلوي و فجوات في الأنسجة الطلائية للانابيب البولية..

ايضا ضمور في خلايا الغدد التكاثرية في ذكور الاسماك و نضوب لكمية البويضات بالنسبة للإناث. وعند استخدام البروبيوتك فقد وجد تناقص في الخلاياالطلائية و عودة التركيب الطبيعي لطبقات الخياشيم ، تجدد و زيادة في طبقة البشرة و الخلاياالعضلية و خلايا الكبد و خلاياالكبدالبنكرياسية. زيادة انقسام أنويةالانابيب البولية (علي نهج طبيعي). تجدد في الخلاياالجرثومية بالنسبة لذكور الاسماك أما بالنسبة للإناث اظهرت امتلاء الحويصلات بالبويضات التكاثرية.

وهذا يوضح أهمية إضافة المحسن الغذائي لرفع مناعة الأسماك وتحفيز الإستجابة المناعية لمقاومة العدوي المختلفة للحفاظ علي حياته وانتاجيته.بالاضافة لكونة محسنا لجوده لحوم اسماك البلطي من الناحبه الميكروبيولوجيه ونسب البروتين والدهون وتحولاتها الخاصة بالفساد والاختبارات الكيميائيه والهستوباثولوجية كما اثبتت التجربة امكانية تطبيق هذا المستحضر و استخدامة فى الاستزراع السمكى المكثف كحل أمثل للسيطره علي مشكلة زيادة الأمونيا والمشاكل المترتبة عليها كأحد المشاكل الجوهرية التى تواجة الاستزراع السمكى .