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TRIALS FOR CONTROL OF *VIBRIO ALGINOLYTICUS* INFECTION IN CULTURED SEABREAM USING PROBIOTICS

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ABSTRACT

Aquaculture is a main contributor to food and nutrition security, while Egypt is a leading producer. Gilt-head seabream (*Sparus aurata*) is a target for intensification as it is a valuable luxury food. Vibriosis is the most significant disease of maricultur representing a great challenge that could hamper the sustainability of the aquaculture industry. In this study, we aimed to evaluate the modulatory effect of combined probiotic "*Lactobacillus fermentum and lactobacillus delbrueckii*" on the experimentally-infected seabream by vibrio spp. One hundred and twenty seabreams were divided into four groups: the first two groups were fed non supplemented diet, while the 3rd and 4th groups were fed probiotic-supplemented and florfenicol-supplemented diets, respectively. Hematological, biochemical, growth and feed utilization parameters and pathological examination were applied to all experimental groups. Our result showed that the probiotic-supplemented diet was able to enhance the growth performance and feed utilization parameters, as well as the survivability of the experimentally-infected seabream. In addition, the heamatological, biochemical parameters and histopathological findings were also improved over the control values.

Keywords: *Lactobacillus fermentum*; *Lactobacillus delbrueckii*; *Sparus aurata*; *Vibrio alginolyticus*; *growth performance, survivability*

INTRODUCTION

Aquaculture as a main source of animal protein, contributes to food and nutrition security. Egypt is a leading producer with a total production of about 1.6 million tons in 2020, from which 350.000 tons come from marine aquaculture (FAO, 2022). Gilt-head seabream (*Sparus aurata*) is one of the most common marine species cultured in Egypt, with a total production of about 38.000 tons in 2020 (GAFRD 2020). As it is a valuable luxury food, it is a target for intensification.

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In aquaculture, the fish is under stressful conditions that tend to disrupt the microbiota homeostasis and consequently increase the risk of infection (Bakry *et al.,* 2023). Noteworthy, diseases and biosecurity challenges hamper the sustainability of the aquaculture industry, while bacterial fish pathogens represent the main cause of diseases in aquaculture. Vibriosis is a septicemic disease targeting the muscle, skin, and haemopoietic organs showing hemorrhagic lesions in the affected organs and is considered the most significant disease of marine and brackish water fishes causing severe mortalities all over the year (Roberts, 2012). Vibriosis is caused by vibrio spp. where, *V. alginolyticus*, *V. vulnificus* and *V. parahaemolyticus* were the most predominant species in Egypt (Aly *et al.,* 2019; Soliman *et al*., 2021; Gobarah *et al*., 2022). Moreover, several members of Vibrio spp. were incriminated as human pathogens.

The aquaculturists, in common, use antimicrobial agents to control fish diseases as therapeutic, prophylactic or metaphylactic agents, but these practices have met some limitations, like the antibiotic resistance development in aquatic bacteria and the residues of public health hazards (Bondad-Reantaso *et al.,* 2023). Noteworthy, as aquaculture is one of the fastest-growing food sectors in the world, sustainable ways for combating fish diseases are needed. Concurrently, several attempts have been performed to meet this need. On the other hand, several probiotics proved encouraging effective alternates to the antibiotics.

Probiotics are live, non-pathogenic bacteria that have the potential to improve microbiota homeostasis, so it's beneficial in enhancing immune response, controlling disease, elevating nutrient utilization, and improving water quality (Sahandi *et al.,* 2019). *Lactobacillus fermentum* improved growth and survival rates and enhanced immune and antioxidant parameters in common carp (*Cyprinus carpio*) (Ahmadifar *et al.,* 2019), while *Lactobacillus delbrueckii* modified the gut microbiota and increased the survival of seabass (*Dicentrarchus labrax, L.*) (Silvi *et al.,* 2008).

This study aimed to evaluate the modulatory effect of *L. fermentum* and *L. delbruekii* on the immunity, antioxidant, liver, and kidney functions, and haematological parameters, as well as the histopathological picture of liver, kidney, and spleen of gilthead seabream exposed to experimental vibrio infection

MATERIALS AND METHODS

1. Ethics of the animal study:

The use of animals in this study complied with the *formal* approval of the Institutional Animal Care and Use Committee of Zagazig University, under approval number ZU-IACUC/2/f/201/2023

2. Fish sampling for vibrio isolation:

A total of 50 moribund and diseased gilthead seabream of 50 ± 10 g showing signs of septicemia, like hemorrhages on dorsal and ventral fins, petechial hemorrhages all over the body were collected during summer mortalities in July from an earthen pond at El-Deeba region in Port-Said & Damietta. They were transported on ice to the wet lab. at Animal Health Research Institute, Zagazig branch. They were dissected and examined, according to Roberts (2012).

3. Isolation and identification of vibrio spp:

Under complete aseptic conditions, loops from the liver, kidney, and spleen were directly streaked upon thiosulphate citrate bile salt sucrose agar (TCBS) and incubated at 28 °C for 24 hours. Each different colony detected on the media was streaked on tryptic soy agar (TSA) supplement and 1.5% (w/v) NaCl plates for purity and identification. Identification of pure bacterial isolates was performed using Grams stain, oxidase test, different concentrations of NaCl (6 & 8 %), and commercial API 20 NE kits.

4. Antibiogram of the isolated bacteria:
Antibiogram of *Vibrio* isolates was assessed by disc diffusion method, as described by Ruangpan and Tendencia (2004). Seven antimicrobial agents were used, which included ciprofloxacin (5µg), tetracycline (30µg), oxytetracycline (30µg), ampicillin (10µg), erythromycin (15µg), florfenicol (30µg), and sulfamethoxazole + trimethoprim (23.75/1.25µg)

5. Experimental infection and control using probiotics:
5.1. Fish: A total of 120 apparently healthy Sea Bream weighing 28 ± 3 g were obtained from a private farm at El-Deeba region during late autumn. For acclimation, fish were distributed equally into 12 glass aquaria of 160 liters capacity (each contained 100 liters of seawater). The aquaria were provided with mechanical filters of 960 L/hour capacity. The water parameters were 22.0 ± 0.5 ˚C, 30 ± 1.7 g L^{-1}, 6.5 ± 0.49 mg L^{-1}, and 8 ± 0.5 for temperature, salinity, dissolved oxygen, and pH respectively, while total ammonia and nitrite were maintained lower than 0.1 mgL^{-1}. Fish were fed isonitrogenous (42%) and isolipidic (15%) commercial Aller Marine 42/15 diet that sized 3mm. The feeding rate was 2.5 % of the fish's body weight divided twice daily / 6 days weekly for two weeks.

5.2. Potential Probiotics:
Commercially available *L. fermentum* and *L. delbruekii* present under the trade name "*lacteol forte"*, produced by Rameda, Giza Governorate, (Egypt) was used in this study. The proposed count per sachet is 10^{10} viable cells. It was selected after Tahoun (2022). The probiotics were incorporated into the diet (according to Ramos *et al.*, 2017) by dissolving the content of one sachet in 50 ml distilled water for 1 hour, then sprayed over 1 kg of feed and left to dry. Finally, the diet is stored at 4 ˚C.

5.3. Experimental design:
The twelve aquaria were divided equally into four groups. Groups 1 & 2 were fed a basal diet, while group 3 was fed a probiotics-supplemented basal diet. The diet of group 4 was supplemented with 15 mg of florfenicol, which was added in the same way as the probiotic. The dose of florfenicol was according to Gaikowski *et al.* (2013). The first three groups were fed the proposed diets for 28 days, while the 4^{th} group was fed the basal diet in the first two weeks, and the florfenicol-supplemented diet in the next two weeks. The feeding regime was the same as during the acclimation period.

5.4. Growth Performance and Feed Utilization Indices:
At the end of the 28-day feeding trial, the final fish weight was recorded and the utilized diets were calculated. Growth performance and feed utilization were calculated as follows:

Weight gain (WG) = final body weight (g) − initial body weight (g)
Feed conversion ratio (FCR) = feed intake (g)/WG (g)
Specific growth rate (SGR) = 100 × [WG /duration of feeding (day)]

5.5. Experimental Infection:
By the end of the feeding trial, the isolated Vibrio was cultivated on brain heart infusion broth (BHI) supplemented with 2% NaCl (W/V) and incubated at a temperature of 25 °C for 24 h. The overnight culture was washed by centrifugation and suspended in PBS (pH 7). The bacterial concentration was adjusted to *0.5 McFarland* as a standard then it was diluted by the addition of PBS (pH 7). Each fish (including the replicates) of groups (2, 3, and 4) was intraperitoneally injected with 0.1 mL of 1.0 x 10^6 CFU (LD_{50} dose of *V. alginolyticus* according to Abdallah *et al.* (2009), while group 1 was inoculated with 0.1 ml of sterile PBS (pH 7). The feeding supplements and regime during the challenge were the same as during the experiment. All injected fish were observed for 7 days post-inoculation, and the daily mortalities were recorded.

5.6. Blood samples:
Each group had two different types of pooled blood samples taken from the caudal vein under strictly sterile conditions. The first 1 ml of blood was drawn on EDTA for haematological analysis. The second blood sample was drawn into a 3-ml clean, dry centrifuge tube without the use of an anticoagulant, allowed to clot at room temperature, and then centrifuged at 3000 rpm for five minutes. For biochemical analysis, serum was sampled, labeled, put in dry, clean tubes with caps, and frozen at -20°C.

5.7. Hematological and biochemical study:
Haemoglobin concentration, packed cell volume, and total leucocytic count were performed manually (Blaxhall and Daisley, 1973), and the erythrocytic count was performed following Feldman *et al.* (2000) guidelines. According to Cole (1986), differential leucocytic counts were determined. According to Murray (1984), ALP (John, 1982), the liver transferases' (alanine aminotransferase, or ALT, and aspartate aminotransferase, or AST) activity were estimated. According to Grant (1987) and Doumas *et al.* (1981), the serum total protein and albumin concentrations were estimated respectively. A mathematical formula was used to determine the serum globulin level by subtracting albumin from the total proteins Doumas *et al.* (1972). Serum urea was determined according to Fawcet and Scott (1960), and the serum creatinine was estimated according to Henry (1974). Superoxide dismutase (SOD) was evaluated according to Spitz and Oberley (1989), and Glutathione peroxidase (GPx) activity was assayed according to Miller and Slebodzinska, (1993). Determination of Immunoglobulins M (IgM) according to Naito (1986)

5.8. Histopathological examination:
Tissue specimens from the liver, kidney, intestine and gills of fish were fixed in neutral buffered formalin 10% for 48 hours, dehydrated in ascending grades of ethanol (70%-100%), cleared in xylene, and embedded in paraffin wax. 5 µm thickness of paraffin sections were obtained by using an automated microtome then stained with routine Hematoxylin and Eosin (H & E) (Suvarana *et al.,* 2018).

5.9. Statistical analysis:
Statistical analysis was performed using the analysis of variance (ANOVA). Duncan's Multiple Range (Duncan, 1955) was used to determine differences among treatment means at a significance level of 0.05. All statistics were run on the computer using the SPSS program SPSS (2004).

RESULTS

1. The Environmental Parameter in a farm at Shatta City, Damietta, Egypt:
The Oxygen, PH, Temp, and Salinity were 3.6 mg/L^{-1}, 9.1, 28 °C, and 26 g/L^{-1} respectively, while Ammonia and Nitrite were more than 1 mg/L^{-1} (these data were provided by the farm administrator).

2. Prevalence of the isolated *V. alginolyticus*:
A total of 42 isolates showed yellow colonies on TCBS, from which 36 isolates grew on TSA supplemented with 8% (w/v) NaCl. All 36 isolates were oxidase-positive, Gram-negative and curved bacilli. API NE commercial kits identified 30 isolates as *V. alginolyticus*.

3. Antibiogram of the isolated *V. alginolyticus* from naturally infected seabream:
Fig. (1) shows the antibiogram of the 30 isolates. The isolated *V.alginolyticus* showed the highest resistance to Ampicillin followed by erythromycin and tetracycline, while the highest sensitivity was noticed to florfenicol followed by ciprofloxacin and oxytetracycline.

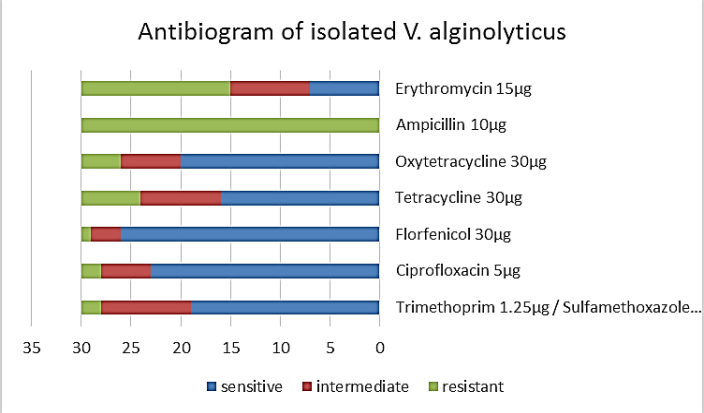

Fig. (1): Antibiogram of the isolated V. *alginolyticus* from naturally infected seabream

4. Growth Performance and Feed Utilization Indices:

Table (1) illustrates the growth performance and feed utilization indices, where group (3) showed the highest levels of WG, FCR, and SGR, while group (4) showed the lowest levels.

5. Cumulative mortalities of experimentally-infected seabream:

The data in Fig. (2) explores the cumulative mortalities in all groups (and their replicates) of seabream challenged with *V. alginolyticus* and PBS. Group 1 (injected with PBS) showed no mortalities, while the other groups showed different levels of mortalities. There were no mortalities on the 1st day in all groups. The highest mortalities were observed in group 2. The deaths in group 3 were the same as in group 4 and began on the 3rd day and stopped on the 5th day.

Table 1: Effect of dietary probiotic and florfenicol on growth performance and feed utilization of seabream:

	Group 1	Group 2	Group 3	Group 4
Initial Body Weight (g)	35.23± 1.11^{a}	34.55 ± 1.09^{a}	35.11± 1.45^{a}	35.31±1.00^{a}
Final Body Weight (g)	45.92± 1.19^{b}	45.17± 1.21^{b}	48.92± 1.55^{a}	45.43± 0.97^{b}
Weight Gain (g)	10.69± 0.85^{b}	10.62± 0.78^{b}	13.81± 0.90^{a}	10.12± 0.61^{b}
Feed Conversion Ratio	2.25± 0.17^{a}	2.26± 0.14^{a}	1.74 ± 0.20^{b}	2.37± 0.11^{a}
Specific Growth Rate (g/d)	38.18± 1.91^{b}	37.93± 1.88^{b}	49.32 ± 2.12^{a}	36.14± 1.79^{b}

Groups with different letters within the same column are significantly different at P < 0.05

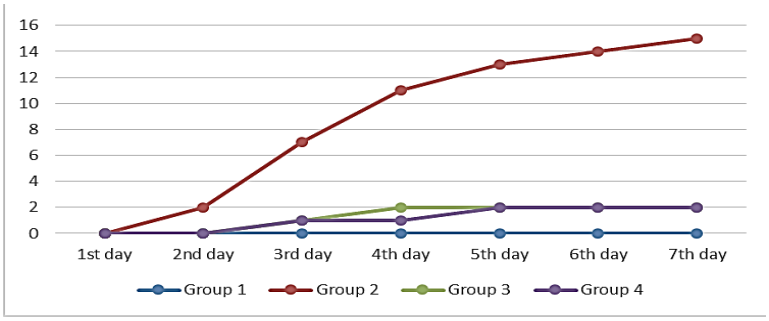

Fig. 2: Cumulative mortalities of experimentally *V.alginlyticus*-infected seabream-fed different diets:- Cumulative mortalities of *Sparus aurata* during experimental infection

6. Hematological parameters:

Results presented in (table 2) showed a significant decrease ($P < 0.05$) in the RBCs count, hemoglobin concentration and packed cell volume in group 2, also leukocytosis, lymphocytosis and monocytosis were

detected compared to group 1. Infected groups (3,4) were treated with probiotic and florfenicol respectively, showed a significant increase (P<0.05) in RBCs count, hemoglobin concentration and packed cell volume compared with the infected non-treated group (2).

7. Biochemical parameters:

Our results in Table (3) revealed a significant decrease in total protein, albumen, and globulin and a significant increase (P<0.05) in AST, ALT, ALP, creatinine and urea in group 2 compared to group 1. Treated groups (3,4) showed a significant decline in levels of SOD and GPX in comparison with group 1, while treated groups (3,4) showed a significant increase in SOD and GPX compared with group 2.

Table 2: Mean values of hematological parameters in Seabream.

Parameters	Group 1	Group 2	Group 3	Group 4
RBCs × 10^6 /µl	3.21±0.14 ^{ab}	2.63±0.20^{c}	3.51±0.07^{a}	3±.04^{b}
Hb (g/dl)	9.52±0.17 ^{b}	7.43±0.31^{c}	10.96±0.18 ^{a}	9.25±0.17 ^{b}
PCV%	30.1±0.46 ^{a}	22.8±1.01^{b}	31.2±0.20 ^{a}	29.93±0.52 ^{a}
WBCs (10^3×mm^3)	10.51±0.24^{c}	14.15±0.86^{a}	9.34±0.25 ^{c}	12.06±0.42 ^{b}
Lymphocytes (10^3×mm^3)	7.82±0.26 ^{cd}	10.96±0.89^{a}	6.67±0.17 ^{d}	9.10±0.38 ^{bc}
Monocytes (10^3×mm^3)	0.35±0.06^{bc}	0.64±0.07^{a}	0.29±0.09^{c}	0.44±0.02^{b}
Neutrophil (10^3×mm^3)	2.29±0.09^{a}	2.48±0.08^{a}	2.34±0.09^{a}	2.41±0.02^{a}
Eosinophil (10^3×mm^3)	0.02±0.01^{b}	0.03±0.05^{a}	0.02±0.01^{b}	0.02±0.01^{b}
Basophil (10^3×mm^3)	0.03±0.02^{b}	0.04±0.01^{a}	0.03±0.01^{b}	0.03±0.03^{b}

RBCs: Red blood cells Hb: Hemoglobin PCV%: Packed cell volume WBCs: White blood cells
Groups with different letters within the same row are significantly different at $P < 0.05$

Table 3: The mean values of biochemical and immunological parameters in seabream.

Parameters	Group 1	Group 2	Group 3	Group 4
T. protein (g/dl)	3.69±0.04^{b}	2.84±0.06^{d}	3.86±0.09 ^{a}	3.28±0.06^{c}
Albumin (g/dl)	1.70 ± 0.11^{b}	1.33 ± 0.07^{c}	2.00 ± 0.10^{a}	1.56±0.10^{bc}
Globulin (g/dl)	1.99 ± 0.06^{a}	1.51 ± 0.02^{d}	1.86 ± 0.08^{ab}	1.72±0.04c
A/G Ratio (g/dl)	0.85 ± 0.08^{b}	0.88± 0.04^{b}	1.08 ± 0.06^{a}	0.91±0.08^{ab}
ALT (U/L)	5.89 ± 0.34^{b}	19.59± 1.1^{a}	4.94 ± 0.41^{b}	6.28±0.49^{b}
AST (U/L)	8.96 ± 0.35^{c}	22.58± 1.02^{a}	8.86 ± 0.33^{c}	11.89±0.19^{b}
ALP (U/L)	28.35 ± 0.49^{b}	48.03± 1.3^{a}	28.18 ± 0.87^{b}	30.5±0.37^{b}
Creatinine (mg/dl)	0.63 ± 0.02^{c}	1.10 ± 0.08^{a}	0.64 ± 0.04^{c}	0.85±0.02^{b}
Urea (mg/dl)	2.59 ± 0.16^{b}	3.45 ± 0.08^{a}	2.44 ± 0.17^{b}	2.78±0.09^{b}
SOD (U/ml)	32.61 ± 1.2^{ab}	15.76 ± 0.72^{c}	37.47 ± 1.3^{a}	28.96±0.82^{b}
GPX (U/ml)	30.57 ± 2.2^{a}	15.00 ± 0.98^{b}	30.35 ± 2.1^{a}	29.82±1.32^{a}
IgM (ng-ml)	187.3 ± 27^{b}	296.00 ± 17.1^{a}	341.30 ± 21^{a}	330±17^{a}

AST: aspartate aminotransferase ALT: alanine aminotransferase ALP: Alkaline phosphatase
SOD: Superoxide dismutase GPX: Glutathione peroxidase
Groups with different letters within the same column are significantly different at $P < 0.05$

8. Histopathological findings:

Degenerative changes mainly fatty degeneration in most hepatocytes, as well as engorged hepatic vasculatures were seen in the liver in the infected group with Vibrio **(Fig. 3A**). While the treated group with antibiotics **(fig.3B)** showed a reduction of degenerated areas with the presence of a small number of vacuolated hepatocytes. In addition, minute infiltrations of round cells within peripancreatic areas were detected. However, the probiotic-supported group

(fig.3C) revealed normal morphology of hepatopancreas with the presence of a few numbers of swelled hepatocytes. The control negative group **(fig.3D)** showed normal histological configurations of the pancreas, hepatic cells and stromal components.

The infected kidney with Vibrio **(fig.4A)** showed a destroyed large number of renal tubules, necrosis of some renal tubular epithelium and hyperemic renal blood vessels. While apparent normal glomerular structures and fewer degenerated tubules were observed in the treated infected group with antibiotics **(fig.4B).** Probiotic-supplemented group **(fig.4C)** revealed normal histology of glomerular corpuscles, renal tubules, and stroma. The control negative group **(fig.4D)** showed preserved morphological structures of renal parenchyma.

The infected intestine with Vibrio **(fig.5A)** showed necrotically and desquamated few intestinal villi, mucous exudate within intestinal lumina and degenerated muscularis layer. However, apparent normal intestinal walls with edematous few villous tips were seen in the treated infected group with antibiotics **(fig.5B).** Probiotic-cured group **(fig.5C)** revealed normal histology of intestinal mucosa, submucosa and musculosa. Moreover, the non-infected group **(fig.5D)** showed normal morphological structures of intestinal enterocytes, lamina propria and muscular layer.

Sections from gills in the infected group with vibrio **(fig.6A)** exhibited hyperplastic epithelial lining gill filaments. The latter were stuck with each other in some areas by lymphocytic aggregates. Hyperemic gill capillaries were also seen. However, the treated infected group with the antibiotic **(fig.6B)** showed apparent normal histological structures in the majority of gills. However, detachment of secondary filaments and fused secondary filaments with mucous exudate were also seen. Probiotic-treated group **(fig.6C)** displayed normal histology of primary and secondary gill filaments beside the presence of hyperemic gill capillaries. Furthermore, the control negative group **(fig.6D)** showed normal morphological structures of the gill arch, gill rackers, and gill lamellae.

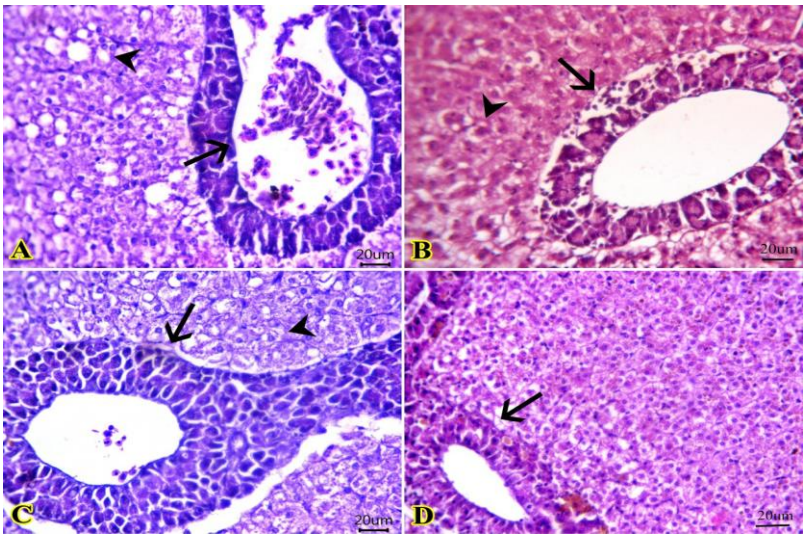

Fig. 3: Photomicrograph of H&E stained sections from hepatopancreas of fish. (Scale bar 20µm) showing: A: fatty degeneration in most hepatocytes (arrowhead) and engorged portal vein (arrow) in the infected group with Vibrio. B: a small number of vacuolated hepatocytes (arrowhead) and minute infiltration of round cells within the peripancreatic area (arrow) in the treated infected group with the antibiotic. C: normal morphology of pancreatic acini (arrow) with the presence of a few numbers of swelled hepatocytes (arrowhead) in the prophylactic group. D: normal histological configurations of the pancreas (arrow), hepatic cells and stromal components in the control negative group.

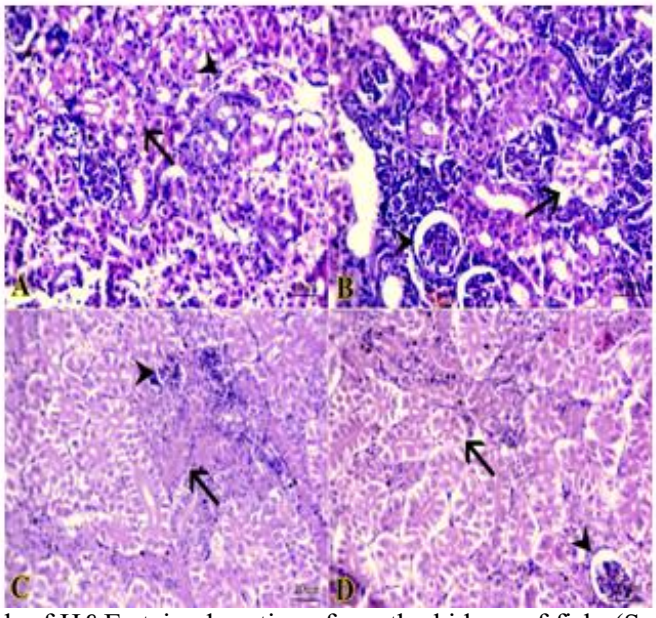

Fig. 4: Photomicrograph of H&E stained sections from the kidney of fish. (Scale bar 20µm) showing: A: destructed necrotic renal tubular epithelium (arrow) and hyperemic renal blood vessels (arrowhead) in the infected group with Vibrio. B: apparent normal glomerular structures (arrowhead) and fewer number of degenerated tubules (arrow) in treated infected group with antibiotic (arrow) in treated infected group with antibiotic. C: normal histology of glomerular corpuscle (arrowhead), renal tubule (arrow) and stroma in the prophylactic group. D: preserved morphological structures of glomeruli (arrowhead) and renal tubule (arrow) in the control negative group.

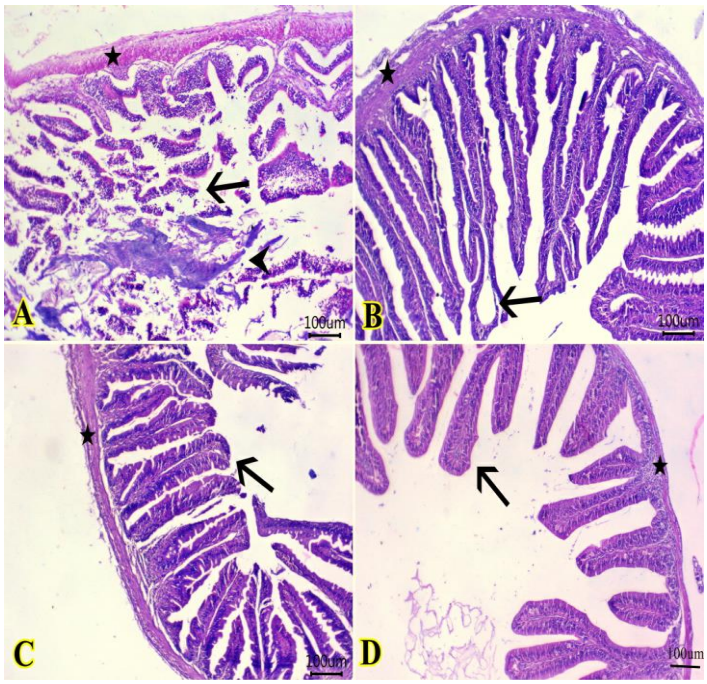

Fig.5: Photomicrograph of H&E stained sections from the intestine of fish. (Scale bar 100µm) showing: A: necrotic and desquamated some intestinal villi (arrow), mucous exudate within intestinal lumina (arrowhead) and degenerated muscularis layer (star) in the infected group with Vibrio. B: apparent normal intestinal walls, muscular layer (star) with edematous some villous tips (arrow) in treated infected group with antibiotic (arrow) in treated infected group with antibiotic. C: normal histology of intestinal mucosa (arrow), submucosa and musculosa (star) in the prophylactic group. D: normal morphological structures of intestinal enterocytes (arrow), lamina propria and muscular layer (star) in the control negative group.

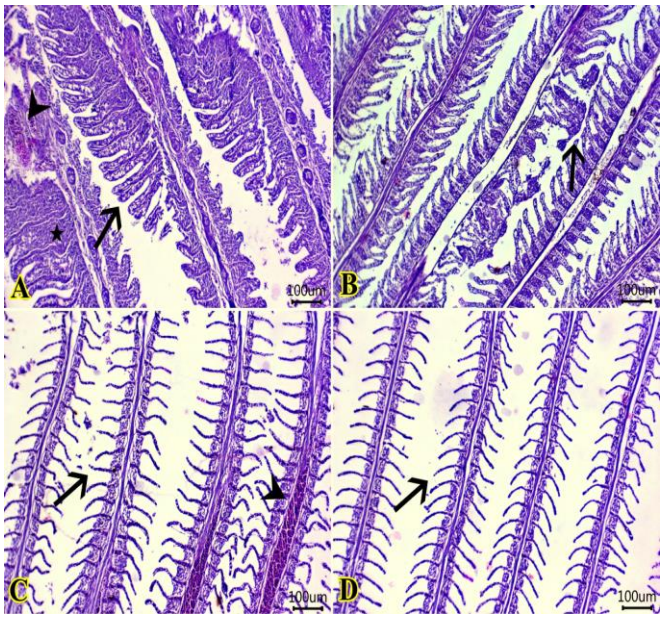

Fig. 6: Photomicrograph of H&E stained sections from gills of fish. (Scale bar 100µm) showing: A: hyperplastic epithelial lining gill filaments (arrow), stuck primary filaments by lymphocytic aggregates (star) and hyperemic gill capillaries (arrowhead) in infected group with Vibrio. B: detachment of some secondary filaments and fused some secondary filaments with mucous exudate (arrow) in the treated infected group with antibiotic (arrow) in the treated infected group with antibiotic. C: normal histology of primary and secondary gill filaments (arrow) beside hyperemic gill capillaries (arrowhead) in the prophylactic group. D: normal morphological structures of gill lamellae (arrow) in the control negative group.

DISCUSSION

Vibrio sp. is considered the main bacterial pathogen threatening marine fish's health, representing a major constraint on mariculture sustainability. *V. alginolyticus* was the predominant isolated *vibrio* Sp. in our study. Concurrently, several studies identified *V. alginolyticus* as a causative agent of mass mortalities in cultured seabream in the Mediterranean region (Kahla-Nakbi *et al.*, 2007; Snoussi *et al.*, 2008; Abdel-Aziz *et al.*, 2013; Aly *et al.*, 2019). In consistency, the environmental parameters were above the permissible levels, which could be a predisposing factor for the infection.

Continuous monitoring of the bacterial-resistance status becomes an essential public health need. The antibiogram of the isolated *V. alginolyticus* showed complete resistance to ampicillin and partial resistance to other antimicrobial agents, which partially agrees with the results of previous studies (Kahla-Nakbi *et al.*, 2006; Snoussi *et al.*, 2008; Abdel-Aziz *et al.*, 2013; Aly *et al.*, 2019). Our findings of the antibiogram of *V. alginolyticus* showed an increase in the status of vibrio-resistance profile, especially for erythromycin and tetracycline, giving rise to increasing the need for more responsible and wise use of antimicrobial agents in the aquaculture sector.

In aquaculture, probiotics enhance fish performance and feed utilization due to the improvement in nutrient digestibility and absorption (Dimitroglou *et al.*, 2011; Martínez Cruz *et al.*, 2012). Actually, our results showed that lactobacillus Sp. was able to improve WG, FCR, and SGR over the control values. In agreement, the seabream-fed probiotic-supplemented diet showed better growth performance (Moroni *et al.*, 2021). The probiotics secrete enzymes, such as proteases, amylases, and lipases that hydrolyze molecules in the fish intestine, which could improve feed utilization (Balcázar *et al.*, 2006; Abd El-Rhman *et al.*, 2009).

Our results showed that the ability of probiotics to protect vibrio-challenged seabream was comparable to florfenicol. The probiotic antibacterial efficacy is attributed to their production of antibiotics, organic acids, hydrogen peroxide, bacteriocins, carbon dioxide and siderophores (Goa *et al.*, 2010).

The application of probiotics as nutritional supplements is one of the most popular techniques used in aquaculture to strengthen aquatic animals' immunological defenses against vibriosis (Ring *et al.*, 2010; Hoseinifar *et al.*, 2020). Probiotics might considerably ($p < 0.05$) enhance levels of RBCs, Hb, and Hct compared to the infected group (2), according to the results of the current study. Our findings are consistent with those of Al-Dohail *et al.* (2011), Talpur *et al.* (2014), and Kumar *et al.* (2015), who demonstrated that probiotics play a role in preserving the health and homeostasis of fish exposed to infections. Additionally, probiotics, according to Mohapatra *et al.* (2014) and Elshaghabee *et al.* (2017), can encourage hematopoiesis by lowering blood cortisol levels, providing necessary micronutrients, particularly B-Group vitamins, and positively modulating the gut flora. In the present study, the infected fish with vibriosis and received the probiotics, as nutritional supplements showed a significant improvement in the WBC and LYM, which proved the immune-stimulatory effects of probiotics and positive effects on the health of seabream that come following El-Bab *et al.* (2022). Dietary probiotic supplementation in infected seabream resulted in a significantly higher total protein, albumin, and globulin than the infected fish. This might be due to the boosted immune responses against vibriosis, as recorded before (Kumar *et al.* 2015; Devi *et al.* 2019; Zhang *et al.* 2022). This demonstrated the probiotics' immune modulatory effects against fish pathogens. Similarly, the rise in IgM activity in the probiotic groups showed the importance of probiotics in stimulating immunological responses in seabream and preserving the structure of lymphoid tissue and B cell survival.

Hepatic enzymes indicated a significant elevation ($p < 0.05$) in group 2 compared to the group 1 and probiotics groups concerning the effect of vibrio infection on hepatic enzymes (AST, ALT, and ALP). There were also significant increases in blood levels of urea and creatinine. According to our study's histopathological findings, this rise may be related to the liver and kidney damage brought on by Vibrio infection, like those of Mohamad *et al.* (2019) and Xie *et al.* (2020).

Antioxidant enzyme activity is utilized to assess the antioxidant status, because it serves as a marker for stressors brought on by ROS formation in fish. According to Jiao *et al.* (2019), excessive ROS causes lipid peroxidation, membrane deterioration, and cell death. In the present study, a drop in the infected non-treated evoked a significant decrease in the levels of SOD & GPX. However, the groups that received dietary probiotics demonstrated a significant increase in the previous parameters. our results approved those of El-Bab *et al.* (2022), who recorded a significant increase in SOD and CAT activities in Seabream-administered dietary *Saccharomyces cerevisiae*. Ghaly *et al.* (2023) reported a significant increase in SOD & CAT in Nile tilapia that received additives of probiotics.

Our results in the vibrio-infected group are similar to the results obtained previously (Diggles *et al.*, 2000; El-Bassiony, 2001; Korun and Timur, 2008). These histo-pathological lesions in the internal organs can be attributed to the septicemic nature of Vibrio infection.

Our results in the *lactobacillus-treated* group were in line with the results obtained by Pooljun *et al.* (2020), who found that hepatopancreas of shrimp fed on *lactobacillus* then challenged with vibrio having normal structure and fewer pathological alterations occur. These findings

were attributed to the action of the polysaccharides (such as α-D-glucan and β-D-glucan) of the yeast cell wall that binds to the bacterial outer membrane, preventing the attachment and colonization of pathogens in the gastrointestinal tract (Ohland and Jobin 2015).

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محاولات السيطرة علي عدوى الفيبريو ألجينوليتيكس فى سمك الدنيس المستزرع باستخدام البروبيتك

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الاستزراع السمكى مساهم أساسى فى الأمن الغذائى. تعد مصر منتج رئيسى لبعض الأسماك. تعتبر أسماك الدنيس هدف للاستزراع السمكى لأنها غذاء فاخر. مرض الفيبريو هو أهم الأمراض التى تصيب المزارع البحرية مما يشكل تحدى قد يعوق استمرارية هذه الصناعة. تم عمل هذه الدراسة لتقييم التأثير المشترك لبروبيوتك لاكتوباسيلس فيرمينتم ولاكتوباسيلس ديلبريكى على أسماك الدنيس المصابة اصطناعيا بميكروب الفيبريو. تم تقسيم مائة وعشرون سمكة دنيس إلى أربعة مجموعات حيث تم تغذية المجموعة الأولى والثانية بعليقة اساسية بينما تم اضافة البروبيوتك والفلوروفينيكول لعليقة المجموعة الثالثة والرابعة على التوالي. وتم تعيين معاملات النمو واستهلاك الغذاء وكذلك دراسة مكونات الدم والكيمياء الحيوية والفحص الهستوباثولوجى فى جميع المجموعات التجريبية. أظهرت النتائج أن النظام الغذائي المدعم بالبروبيوتيك قادر على تحسين أداء النمو ومعايير الاستفادة من العلف وكذلك قدرة الدنيس على البقاء على قيد الحياة إلى جانب التحسن في مكونات الدم والكيمياء الحيوية الصورة التشريحية المرضية مقارنة بالأسماك المستخدمة كضبط للتجربة.