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Effects of commercial and fish isolated gut *Bacillus* strains supplementation on the performance of *Oreochromis niloticus* challenged with *Aeromonas hydrophila*.

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Abstract

Apparently healthy *Oreochromis niloticus* (n = 225) were used to investigate the effect of commercial probiotic (*B. subtilis*) or fish gut isolated *Bacillus* strains (*B. amyloliquefaciens*; 7 H N and *B. cereus*; 29 H N) on growth performance, immune response, some serum biochemical parameters and disease resistance against *Aeromonas hydrophila* infections. They were randomly assigned to four treatments with three replicates per treatment (45 fish/ treatment; 15 fish/replicate except control group has six replicates). The experimental fish were fed with diets supplemented with 0 (control), 1 x 10^6 CFU g^{-1} fed commercial *B. subtilis* or gut isolated *B. amyloliquefaciens* (7 H N) and *B. cereus* (29 H N) for 2 months then bacterial challenge were performed at the end of the experiment. Fish fed gut isolated *B. cereus* (29 H N) had the highest marked ($p<0.05$) influence on the productive performance parameters among treated groups. Furthermore, the highest levels of nitric oxide, Immunoglobulin (IgM) and lysozyme were found in treated groups. Infected fish fed on gut isolated *B. cereus* had a positive effect on ALT, SOD activity while creatinine and urea were marked reduction compared with other dietary treatments. It could be inferred that gut isolated *Bacillus* has been suggested as growth promoter, immune-stimulant in *O. niloticus* and increase protection against *A. hydrophila* infection. Among the two isolated *Bacillus* species, dietary supplementation with the *B. cereus* had the highest performance in *O. niloticus* compared with the commercial *B. subtilis*.

Keywords: *Bacillus*, *O. niloticus*, Growth, Immunity, Biochemistry, *Aeromonas hydrophila*.

INTRODUCTION

Aquaculture industry is of a great importance, as a fastest growing production occupied 60% of fish production which offers cheap high-quality omega-3 fatty acids enriched animal protein used for human consumption **(Reda *et al.*, 2018).** The major outbreak causing severe economic losses could occur due to the exposure to microbial diseases in intensive fish aquaculture **(Aly, 2013)**. The dealing with bacterial diseases depends mainly on the abuse of antibiotics and chemicals that negatively affect fish, human and environment, leading to the development of antibiotic resistance, disturbance to gastrointestinal microbial population and immune suppression **(Harikrishman *et al.*, 2010; Reda *et al.*, 2013)**. Consequently, several probiotic "live beneficial microorganisms" is expected to become an eco-friendly potential alternative growth promoters for chemotherapeutics **(Reda *et al.*, 2018**); used to control fish disease and to maintain a healthy microbial aquatic environment **(Kavitha *et al.*, 2018)**.

The most probiotics used in fish farms are commercial products isolated from non-fish sources; their existence could be unreliable in aquatic ecosystem **(Azad and AL-Marzouk, 2008)**. The beneficial effects of indigenous probiotics isolated from the gut of fish as growth promoters can be exerted through improving intestinal morphology (epithelial barrier; adhesion to intestinal mucosa), providing digestive enzymes, improving gut microbiota, competing the pathogenic bacteria by inhibitory substances production and enhancing the immune response, and inducing the pro-inflammatory cytokines **(Das *et al.*, 2013; Reda and Selim, 2015; Selim and Reda, 2015).**

Among the indigenous probiotics, *Bacillus* is aerobic, Gram positive, heat stable spore forming bacteria **(Nicoholson *et al.*, 2000; Hong *et al.*, 2005)** and one of the most commonly used probiotics as growth promoters in aquaculture **(Selim and Reda, 2015)**. Besides, *Bacillus* species has antibacterial activities, can survive in high acidic media of the stomach or high concentration of bile, able to colonize in the gut and can produce digestive enzymes **(Reda *et al.*, 2017)**.

Some previous study isolated and identified some indigenous probiotic strains from the gut of *Clarias gariepinus* **(Reda *et al.*, 2017 and Reda *et al.*, 2018)** or *Labeo calbasu* **(Kavitha *et al.*, 2018)**. As of now, the potential effects of gut isolated probiotics and applications in fish as growth promoter remain elusive. The current trial was delineated to investigate the improvement of productive performance, some immune response, serum biochemistry and disease resistance against *Aeromonas.*

hydrophila infections of *O. niloticus* by commercial or gut isolated probiotics dietary supplementation.

MATERIAL AND METHODS

Experimental fish:

A total number of 225 apparently healthy *O. niloticus* with an average body weight 25.5±0.5 g were purchased from Central Laboratory for Aquaculture reserch, Abassa Fish Farm at Sharkia province. Fish were kept in glass aquaria filled with 90 L de-chlorinated fresh water. The water temperature, dissolved oxygen, pH, ammonium and nitrite were measured and found to be 27 ± 2° C, 5.4 mg/l, 7.2, 0.20 mg/l and 0.02 mg/l respectively.

Probiotic supplementation:

Commercial Biomin probiotic (Natural growth promoter):- used as a commercial probiotic, each 1gm contains *Bacillus subtilis*, 1 x 10^6 CFU g^{-1}.it is Produced by Biomin Holding GmbH, Industries trasse 21, A-3130 Herzogenburg, Austria., imported by Dakahlia Campany Egypt. Gut isolated *B. amyloliquefaciens* (7 H N) and *B. cereus* (29 H N). Two strains of gut isolate probiotic bacteria, *B. amyloliquefaciens* (7 H N) and *B. cereus* (29 H N), were previously isolated from the intestine of *C. gariepinus*, were identified by 16 S rRNA gene sequencing and maintained in tryptic soya agar slopes at 4°C. Different sequences of 16 S rRNA were submitted to the Gene bank database and accession numbers were KX015882 and KX015885, respectively were kindly supported by d.rasha reda fish disease department **(Reda *et al.,* 2018).** The previously isolated *Bacillus* species were screened for activity against fish pathogens, safety, resistance to acidic pH and tolerance, antibiotic susceptibility, extra-cellular amylase and protease production **(Reda *et al.,* 2018).** One mL of the culture (24 h) of each isolate was centrifuged at 3000 rpm for 30 min at 4°C. The pellets were washed by sterilized saline then were centrifuged at 3000 rpm for 5 min. The final concentration of each probiotic isolate was adjusted to 10^{10} CFU/ml in saline using McFarland standard tube.

Feeding and experimental design:

The diets were prepared at Fish Research Center, Faculty of Veterinary medicine, Zagazig University, Egypt. The fish were randomly assigned into 4 experimental treatments with 3 replicates/treatment (45 fish/ treatment; 15 fish/replicate except control group has six replicates). Experimental treatments supplemented with probiotics in diets at rate 0 (control treatment), 1 x 10^6 CFU g^{-1} feed commercial *B. subtilis* (treatment 2), 1 x

10^6 CFU g^{-1} feed gut isolated *B. amyloliquefaciens* (7 H N) (treatment 3) and 1 x 10^6 CFU g^{-1} feed gut isolated *B. cereus* (29 H N) (treatment 4) for 2 months**.** It was prepared by mechanical mixing probiotics with the basal diet ingredient table 1&2, and then finally pelleted. The pellets were dried at room temperature (26ºC for 48 h) and stored in a refrigerator at 4ºC until use. It contained (2940 kcal/kg ME and 30.80% CP) in the form of dry pellets and prepared to fulfil the nutrient needs of Nile tilapia **(NRC, 1993).** Feedstuffs used in diets preparation were examined according to **A.O.A.C. (2002)**. All fish were provided with their diets at a level of 3% of body weight three times daily. The feeding period lasted for 2 months and the growth performance indicators were measured.

At the end of feeding period, the control group was subdivided into 2 equal groups (negative (G1) and positive infected (G2) control group) so trial become 5 equal groups, each group contain 45 fish and make challenge test.

Challenge test:

After 60 days of feeding trial, all groups except negative control group (G1) were challenged with intraperitoneal injection of 0.1 ml of pathogenic *A. hydrophila* (10^8 CFU mL^{-1}; adjusted by using McFarland standard tubes) previously isolated from moribund fish and confirmed to be pathogenic **(Talpur and Ikhwanuddin, 2012).**

Challenged fish were observed for clinical signs and mortality for 14 days. Any dead fish were subjected immediately to post-mortem examination and routine bacteriological examinations.

Sampling:

Blood samples were collected at 3 days post-challenge for evaluation of some immunological parameters and assessment of some biochemical parameters. Blood samples were taken without EDTA and were centrifuged at 3000 rpm for 15 minutes for serum separation.

Growth Performance Parameters:

The fish were weighed at the beginning and after 2 months feeding. Average body weight (BW), Body gain (BWG), body gain percent (BG %), specific growth rate (SGR) and feed conversion ratio (FCR) were determined according to **(Windell *et al.,* 1978; Merrifield *et al.,* 2011**, **Jauncey and Ross, 1982; Zehra and Khan, 2011**; **Siddiqui *et al.,* 1988**, respectively).

Table 1. Chemical composition of feedstuffs used in formulation of the basal experimental diets.

Ingredient	Nutrient (% as fed basis)					
	DM	CP	EE	CF	Ash	NFE (calculated)
Yellow corn	89.00	8.75	3.70	2.20	1.20	73.15
Wheat flour	88.90	12.80	2.50	1.50	1.60	70.50
Soybean meal (44 %)	90.00	43.70	1.80	6.10	6.50	31.90
Fish meal (65%)	94.80	63.40		8.70	0.7	20.70
Poultry by-product meal	92.60	60.30	12.70	2.10	14.70	2.80

(DM= Dry matter, CP= Crude protein, EE= Ether extract, CF= Crude fiber and NFE= Nitrogen free extract).

Table 2. Chemical composition of the experimental diets.

Parameters	Experimental basal diet
Yellow corn	35.00
Wheat flour	10.00
Soybean meal (44 %)	18.00
Fish meal (65%)	16.00
Poultry by-product meal	14.00
Vegetable oil	5.50
Vitamins and Minerals mixture*	1.50
Calculated composition	
DM (%)	84.37
CP (%)	30.79
EE (%)	10.26
CF (%)	2.42
Ash (%)	7.12
NFE (%)	38.99
DE (Kcal/ kg diet)**	2944.41

* Vitamin and Mineral mixture (alfakema):- Each 1 kg contains:-Vit. A 580000 I.U, vit.D3 8600 I.U, vit.E. 720 mg, vit. K3 142 mg, vit C 0.1 mg, vit B1 58 mg, vit B2 34 mg, vit. B6 34 mg , vit.B12 58 mg , Folic acid 86 mg , Pantothenic acid 8 mg , Manganese sulfate 65 mg , Zinc methionine 3000 mg , Iron sulfate 2000 mg , Copper sulfate 3400 mg , Cobalt sulfate 572 mg , Sodium selenite 25 mg, Calcium iodide 25 mg, Calcium carbonate (Carrier substance) till 1000 gm.

** digestible energy calculation based on values of protein 3.5 kcal/gm, fat 8.1 kcal/gm, NFE 2.5 kcal/gm **(Santiago *et al.*, 1982).**

(DM= Dry matter, CP= Crude protein, EE= Ether extract, CF= Crude fiber and NFE= Nitrogen free extract).

Determination of some immunological and biochemical parameters:

Nitric oxide (NO) level was detected as the method described by **Rajaraman *et al.* (1998)**. Lysozyme activity was detected according to **Parry *et al.* (1965)**. Immunoglobulin (IgM) was measured by an ELISA according to **Fuda *et al.* (1991).** Alanine aminotransferase (ALT) was assessed as described by **Reitman and Frankel (1957).** Serum creatinine was assessed by using the method of **Henry (1974).** Serum superoxide dismutase activity was determined using the method of **(Sheikh *et al.*, 2009).**

Statistical analysis:

Data was analyzed by one way ANOVA using computerized SPSS (version 23; IBM Corp., Armonk, NY) statistical software package; LSD (Least significance difference) test was used to separate significance means **(Snedecor and Cochran, 1982).** Alternations among group means were compared using Duncan's multiple range tests **(Duncan, 1995)**. Statement of statistical significance taken as $p<0.05$.

RESULTS AND DISCUSSION

In the present study, the feeding commercial *B. subtilis*, gut isolated *B. amyloliquefaciens* and gut isolated *B. cereus* supplemented diets for 60 days had a marked ($p<0.05$) influence on total final BW, body gain, body gain %, specific growth rate % and a marked ($p<0.05$) improve or decrease in FCR of *O. niloticus*. Fish fed gut isolated *B. cereus* (29 H N) had the highest marked ($p<0.05$) influence on the productive performance parameters among treated groups Table 3. This strong beneficial effect on productive performance for commercial and gut isolated probiotics could be attributed to promoting nutrients digestibility and absorption; enhancing the synthesis of vitamins, cofactors and digestive enzymes; the depression of pathogenic microbial agents that hinder growth; detoxify the harmful substance in feed or increase the intestinal villus heights **(Reda and Selim, 2015; Reda *et al.*, 2018).** In the present investigation, dietary inclusion of commercial and gut isolated probiotics had a strong effect on the growth performance of fish. These results agreed with some reports showing that the fish productive performance was positively affected by dietary supplementation of commercial *Bacillus* probiotics **(Reda and Selim, 2015)** or gut isolated *Bacillus* probiotics (**Reda *et al.*, 2018)**. In contrast to our findings, growth parameters for fish fed commercial *Bacillus* probiotics **(Zhou *et al.*, 2010)** or gut isolated *Bacillus* probiotics (**Albuquerque *et al.*, 2013)** was not significantly affected.

Table 3: Effect of the dietary commercial and gut isolated probiotics supplementation for 60 days on growth performance parameters of *O. niloticus*.

Parameters	Experimental groups			
	G1	G2	G3	G4
Initial BW (g)	25.80 ±0.06[a]	26.23 ±0.35[a]	26.27 ±0.27[a]	25.63 ±0.09[a]
Final BW (g)	35.58 ±0.39[c]	39.11 ±0.32[b]	39.18 ±0.77[b]	40.80 ±0.12[a]
Body gain (g)	9.78 ±0.33[c]	12.88 ±0.57[b]	12.91 ±0.75[b]	15.17 ±0.03[a]
Body gain (%)	37.90 ±1.20[c]	49.10 ±2.73[b]	49.14 ±2.94[b]	59.18 ±0.14[a]
Specific growth rate (%)	0.53 ±0.01[c]	0.66 ±0.03[b]	0.67 ±0.03[b]	0.77 ±0.01[a]
Feed intake (g)	23.39 ±1.03[a]	22.19 ±2.16[a]	21.84 ±0.44[a]	21.89 ±0.46[a]
Feed conversion ratio	2.39 ±0.03[a]	1.72 ±0.09[b]	1.69 ±0.01[b]	1.44 ±0.11[c]

Values are represented as the mean±SE. Different superscript letters within-row denote significant difference ($p<0.05$). G1: was fed a basal diet, G2: was fed a basal diet supplemented with commercial *B. subtilis*, (6 H N; 1 x 10^6 CFU g^{-1} feed), G3: was fed a basal diet supplemented with gut isolated *B. amyloliquefaciens*, (7 H N; 1 x 10^6 CFU g^{-1} feed), G4: was fed a basal diet supplemented with gut isolated *B. cereus*, (28 H N; 1 x 10^6 CFU g^{-1} feed).

The non-specific immune system of fish is considered to be the first line of defense against invading pathogens. Nitric oxide, IgM and lysozyme are important indices of non-specific immunity in fishes. Our results revealed that the highest levels of nitric oxide, IgM and lysozyme were found in treated groups which fed either commercial or gut isolated probiotics supplemented diets compared with control table ,4. Fish diet supplemented with gut isolated *B. cereus* (29 H N) had the highest marked (*$p<0.05$*) influence on the non-specific immune system among treated groups. Similarly, the administration of probiotics can improve immunity and protect against several pathogens in many fishes **(Gupta *et al.*, 2014).** In contrast to our findings, immune response for fish fed *Bacillus* probiotics (**Heo *et al.*, 2013)** was not significantly affected. The greater immune response in probiotic-supplemented diets might be due to

greater production of antimicrobial substances by probiotic bacteria or due to the natural immune components of the fish themselves, including protective proteins (globulin), lysozyme, nitric oxide, immunoglobulins, activation of proteins of the complement system and cytokines **(Reda and Selim, 2015).**

Nitric oxide (NO) is serum potent bactericidal reactive oxygen that is produced primarily by macrophages following stimulation with a variety of agents, such as microbial components and cytokines **(Campos-Perez *et al.*, 2000)** and showed a variety of biological functions as microbicidal and tumoricidal activity, and a range of immunopathologies **(Saeij *et al.*, 2002)**. In present study results, the highest marked levels of nitric oxide were noticed in infected commercial (*B. subtilis*) or infected gut isolated probiotics (*B. amyloliquefaciens* and *B. cereus*) supplemented diets which indicate that the non-specific immune system was enhanced in the fish. Also, in accordance with our results, serum nitric oxide reported from *A. hydrophila* infected fish supplemented with commercial *Bacillus* probiotics **(Selim and Reda, 2015; Liu *et al.*, 2017)** or gut isolated *Bacillus* probiotics (**Reda *et al.*, 2018)** was significantly affected.

Table 4: Effect of the dietary commercial and gut isolated probiotics supplementation on some immunological parameters of *A. hydrophila* infected *O. niloticus*.

Parameters	Experimental groups				
	G1	G2	G3	G4	G5
Nitric oxide (µg /ml)	26.21 ±0.93^{c}	21.10 ±0.51^{d}	38.07 ±1.10^{b}	40.43 ±0.43^{ab}	41.93 ±1.18^{a}
IgM (µg /ml)	0.30 ±0.02^{c}	0.20 ±0.02^{d}	0.58 ±0.02^{b}	0.71 ±0.004^{a}	0.74 ±0.02^{a}
Lyzozyme (µg /ml)	0.28 ±0.01^{c}	0.22 ±0.01^{d}	0.45 ±0.0.02^{b}	0.49 ±0.01^{b}	0.56 ±0.02^{a}

Values are represented as the mean±SE. Different superscript letters within-row denote significant difference (p<0.05). G1: was fed a basal diet, G2: was fed a basal diet and infected with *A. hydrophila* bacteria, G3: was fed a basal diet supplemented with commercial *B. subtilis* (1 x 10^{6} CFU g^{-1} feed) and infected with *A. hydrophila* bacteria, G4: was fed a basal diet supplemented with gut isolated *B. amyloliquefaciens* and infected with *A. hydrophila* bacteria, (7 H N; 1 x 10^{6} CFU g^{-1} feed), G5: was fed a basal diet supplemented with gut isolated *B. cereus*, (28 H N; 1 x 10^{6} CFU g^{-1} feed) and infected with *A. hydrophila* bacteria.

IgM is the main soluble forms immunoglobulin present in fish **(Watts *et al.,* 2001).** The soluble Immunoglobulin (IgM) forms which are present in the blood and other fluids play a role as an immune effector molecule **(Ross *et al.,* 1998)**. Our study showed marked increasing serum IgM of *A. hydrophila* infected fish supplemented with commercial (*B. subtilis*) or gut isolated probiotics (*B. amyloliquefaciens* and *B. cereus*) supplemented diets when compared to control. On similar ground of our results, serum levels of IgM was significantly improved either in fish fed diets supplemented with commercial *Bacillus* probiotics **(Kamgar *et al.,* 2013; Selim and Reda, 2015)** or gut isolated *Bacillus* probiotics (**Reda *et al.,* 2018)**.

The serum lysozyme activity is considered as a defence barrier against bacterial pathogens thus resulting in the reduction of disease **(Misra *et al.,* 2006).** Lysozyme is an indispensable bactericidal cationic enzyme that hydrolyzes the peptidoglycan layers of bacterial cell walls by splitting glycosidic bonds between N-acetylmuramic acid and Nacetylglucosamine and is increased in the sera of fish during infection with various variable microorganisms **(Alexander and Ingram, 1992)**. Lysozyme is produced by leucocytes, mainly neutrophils and macrophages, and reacts against gram-positive and some gram-negative bacteria **(Saurabh and Sahoo, 2008)**. The present trial revealed a marked elevation in **s**erum lysozyme activity of *A. hydrophila* infected fish supplemented with commercial *B. subtilis*; gut isolated *B. amyloliquefaciens* and *B. cereus* supplemented diets when compared to control. Probiotics can cause increase the IgM, lysozyme and produce different cytokines in the fish so stimulate the immunity system of the fish's stomach through increasing the cells of the immunoglobulin and acidophil granulocyte **(Hoseinfar and Pooramini, 2007)**. The process of the production of the immunoglobulins in the fish is the occurrence of a collection of the reactions among the antigen presenting cells, the activated T helper cells and interleukins which stimulates the B lymphocytes which produce the plasma cells as a result of the stimulation which are able to secrete the immunoglobulin **(Tavakoli and Akhlaghi, 2009).** Our result agrees with some reports stated that the **s**erum lysozyme activity of *A. hydrophila* infected fish was positively affected by dietary supplementation commercial *Bacillus* probiotics **(Kamgar *et al.,* 2013; Selim and Reda, 2015)** or gut isolated *Bacillus* probiotics (**Reda *et al.,* 2018)**. In contrast to our results, serum lysozyme activity for fish supplemented with commercial probiotics was significantly decreased **(Das *et al.,* 2013)** or was not significantly affected **(Cha *et al.,* 2013)**.

In the present study, there were positive differences between dietary treatments and control in resistance to diseases table5. This may be returned to the ability of *Bacillus* spore to survive, transit cross gastrointestinal tract (GIT), resist GIT environments, germinate and vegetate with heterologous antigen expression before being excreted **(Duc *et al.*, 2003)**. These results agreed with some studies showing that the survival rate of *A. hydrophila* infected fish was marked influence by dietary supplementation of commercial *Bacillus* probiotics **(Das *et al.*, 2013)** or gut isolated *Bacillus* probiotics (**Liu *et al.*, 2017; Reda *et al.*, 2018)**. In contrast to present findings, survival rate for fish fed *Bacillus* probiotics (**Cerezuela *et al.*, 2012)** was not significantly affected.

Serum ALT, creatinine and urea are considered important parameters to evaluate unconventional feedstuffs and new feed additives for its proper additional level **(Diaz *et al.*, 2003)**. The present results are showing significant ($p<0.05$) reduction for serum ALT value between infected treated fish groups, table 5. Infected fish fed on gut isolated *B. cereus* had a positive effect on ALT. This reduction in probiotics groups might be attributed to their roles in improvement of liver histology. In line with our findings, the **s**erum ALT activity of *A. hydrophila* infected fish was a significant decrease by dietary inclusion of *Bacillus* probiotics **(Kamgar et al., 2013).** While in our study, there was a significant increase in ALT level was noticed in infected control group. Elevated levels in ALT value may indicate degeneration, necrosis, and destruction of the liver and kidney due to cellular damage**.**

Creatinine and urea in fish can be used as a rough index of the glomerular filtration rate **(Hernandez and Coulson, 1967)** and urea is produced by liver and excreted by kidney **(Stoskoph, 1993)**. In our trial, creatinine and urea of *A. hydrophila* infected fish were marked reduction by dietary supplementation of commercial or gut isolated probiotic bacteria. The reduction of serum urea in probiotics groups might be attributed to their roles in improvement of kidney histology. These results agreed with some studies showing that the creatinine and urea of *A. hydrophila* infected fish was marked influence by dietary supplementation of *Bacillus* probiotics bacteria **(Kamgar *et al.*, 2013)**.

In our study, there were positive differences between dietary treatments and control in serum SOD activity. These results agreed with some studies showing that the serum SOD activity for *A. hydrophila* infected fish was marked increase by dietary supplementation of commercial *Bacillus* probiotics **(Cha *et al.*, 2013)** or gut isolated *Bacillus* probiotics (**Reda *et al.*, 2018)**. In contrast to our findings, serum SOD

activity for *A. hydrophila* infected fish fed *Bacillus* probiotics (**Thy *et al.*, 2017)** was not significantly affected.

Table 5: Effect of the dietary commercial and gut isolated probiotics supplementation on survival rate, serum levels of ALT, creatinine, urea and SOD of *A. hydrophila* infected *O. niloticus*.

Parameters	Experimental groups				
	G1	G2	G3	G4	G5
Survival rate (%)	77.30	65.20	94.60	95.70	96.80
ALT (IU/L)	**43.94** ±0.51^{b}	**45.91** ±0.47^{a}	**36.89** ±0.50^{c}	**35.90** ±0.49^{c}	**33.90** ±0.48^{d}
Creatinine (mg/ dl)	**0.44** ±0.01^{b}	**0.51** ±0.01^{a}	**0.37** ±0.0.02^{c}	**0.32** ±0.02^{d}	**0.31** ±0.02^{d}
Urea (mg/ dl)	**17.69** ±0.38^{b}	**26.76** ±0.83^{a}	**14.33** ±0.61^{c}	**13.53** ±0.20^{cd}	**12.12** ±0.41^{d}
SOD (ug/ml)	**0.08** ±0.01^{c}	**0.04** ±0.01^{d}	**0.20** ±0.0.02^{b}	**0.23** ±0.02^{b}	**0.32** ±0.02^{a}

Values are represented as the mean±SE. Different superscript letters within-row denote significant difference (p<0.05). G1: was fed a basal diet, G2: was fed a basal diet and infected with *A. hydrophila* bacteria, G3: was fed a basal diet supplemented with commercial *B. subtilis* (1 x 10^{6} CFU g^{-1} feed) and infected with *A. hydrophila* bacteria, G4: was fed a basal diet supplemented with gut isolated *B. amyloliquefaciens* and infected with *A. hydrophila* bacteria, (7 H N; 1 x 10^{6} CFU g^{-1} feed), G5: was fed a basal diet supplemented with gut isolated *B. cereus*, (28 H N; 1 x 10^{6} CFU g^{-1} feed) and infected with *A. hydrophila* bacteria.

CONCLUSION

It could be concluded that addition of commercially or gut isolated prepared *Bacillus* species to *Oreochromus niloticus* diets in a dose of 1×10^{10} CFU/g had significantly additive benefit in improved growth performance, immune status, serum antioxidants and functions of liver and kidney. The supplementation of *Bacillus* probiotics increased the survivability percentages after inoculation of fish with *A. hydrophila*. Based on our results, it seems that the gut isolated freshly added probiotic; *B. cereus* had better performance than commercially prepared *B. substilis*. Finally, keeping in view the ecofriendly nature, other beneficial effects of gut isolated freshly added probiotic; it can effectively

provide an alternative to the use of antibiotic growth promoter in the aquaculture industry.

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تاثير الباسلس التجاريه والمعزوله من امعاء الاسماك علي نمو ومناعه اسماك البلطي النيلي المصابه بالايروموناس هيدروفيلا

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الملخص العربي

أجريت هذه الدراسة لتقييم التأثير المحتمل للنمو والتأثيرات المناعية والبيوكيميائية من تغذية البروبيوتيك التجاري(باسيليس ساتلس) أو البكتيريا المعزولة من الأمعاء (*بسلس اميلوليكيوفاشنس وبسلس سيريس*) ومقاومة الأمراض في البلطي النيلي ضد عدوى *الإيروموناس هيدروفيلا*.

أجريت هذه الدراسة على عدد ٢٢٥ سمكة من أسماك البلطي النيلي قسمت إلى ٤ مجموعات متساوية .

وكان تصميم التجربة على النحو التالي:-

المجموعة الاولي: غذيت علي عليقة ضابطة بدون إضافات أو عدوى.

المجموعة الثانية: غذيت علي عليقة ضابطة أضيف إليها باسيليس ساتلس التجاري (١ x ١٠١٠ وحدة تكوين المستعمرة البكتيرية / جرام).

المجموعة الثالثة: غذيت علي عليقة ضابطة أضيف إليها باسيليس *اميلوليكيوفاشنس* المعزولة من الأمعاء (١ x ١٠١٠ وحدة تكوين المستعمرة البكتيرية / جرام).

المجموعة الرابعة: غذيت علي عليقة ضابطة أضيف إليها باسيليس *سيريس* المعزولة من الأمعاء (١ x ١٠١٠ وحدة تكوين المستعمرة البكتيرية / جرام).

بعد ٦٠ يومًا من بدء التغذية تم تقسيم المجموعه الضابطه لمجموعتين موجبه وسالبه، يتم العدوى بحقن ٠,١ مل من ميكروب *الإيروموناس هدروفيلا* لجميع المجموعات.

اظهرت مجموعات الاسماك التي غذيت علي باسيليس ساتلس التجاري ، *بسلس اميلوليكيوفاشنس* و باسيليس *سيريس* المعزولين من الأمعاء زيادة معنوية في إجمالي وزن الجسم النهائي، معدلات النمو ومعدلات التحويل الغذائي مقارنة مع مجموعات الاسماك الأخرى . وكانت مجموعة الاسماك التي غذيت على باسيليس *سيريس* المعزولة من الأمعاء أعلى تأثير ملحوظ على الأداء الإنتاجي بين المجموعات المعالجة.

١- اظهرت النتائج زيادة معنوية في قيم الأكسيد النيتريك، الجلوبولين المناعي والليزوزيم في مجموعات الاسماك التي غذيت علي البروبيوتيك التجاري او المعزول من الامعاء وكانت مجموعة الاسماك التي غذيت على باسيليس *سيريس* المعزولة من الأمعاء كان لها أعلى تأثير ملحوظ على نظام المناعة غير المحدد بين المجموعات المعالجة.

. استناداً إلى نتائجنا ، يبدو أن باسيليس *سيريس* المعزول من الامعاء اعطى أداء أفضل من باسيليس *ستليس* التجاري. وأخيرا ، مع الأخذ في الاعتبار ;كونه صديقا للبيئة ، والآثار المفيدة الأخر للبروبيوتيك المعزولة من الأمعاء يمكن أن توفر بشكل فعال بديل للمضادات الحيوية المستخدمة كمحفز نمو في صناعة وتربية الأحياء المائية.