EFFECT OF PLANT BIOPSIES *LACTOBACILLUS PLANTARUM VSG3* IMPROVES ON IMPROVE RESISTANCE OF FRESHWATER FISH TO DISEASES

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Abstract

In the present study, the immunological efficacy of cellular components from the potential probiotic bacteria *Lactobacillus plantarum* VSG3 was evaluated in Nile tilapia. Fresh water fish, Nile tilapia weighted average 100gm ±1gm were immunized intraperitoneally with 0.1 mL phosphate-buffer solution (PBS) containing *Lactobacillus plantarum* VSG3 at 10^4, 10^6, 10^8 and 10^{10} Colony Forming Units (CFU)/mL were tested by intraperitoneal (i.p) injection. Nile tilapia were challenged 40 days after vaccination. Nile tilapia injected with 0.1 mL served as the control. The results revealed that administration of cellular components significantly increased the activity of serum lysozyme activity and phagocytosis, hematology, serum total protein and immunoglobin IgM after the experimental period. Serum glucose, lipids, cholesterol, triglycerides, AST, ALT, ALP, uric acid, urea and creatinine levels were significantly lower in experimental groups than in the control. The capacity of lymphocyte proliferation and macrophages phagocytosis were significantly increased in four treatment groups as compared with the PBS control group. The hematological, immunological IgM, Lysozyme activity and serum biochemical parameters were significantly increased in four treatment groups as compared with the control group. The number of erythrocytes was higher in Nile tilapia treated with *Lactobacillus plantarum* VSG3 than control groups. After challenge, total number of thrombocytes was higher in fish that received the greatest dose of vaccine. Total number of leukocytes and the number of lymphocytes showed the highest values in vaccinated Nile tilapia. Increased number of monocytes in vaccinated than saline-injected fish was observed. These results indicate that cellular components of probiotic bacteria can influence immune responses, enhance disease protection, and stimulate immune-related gene expression in Nile tilapia. So, the immunity and

health of Nile tilapia treated with doses of *Lactobacillus plantarum* VSG3 at 10^4, 10^6, 10^8 and 10^{10} Colony Forming Units (CFU)/mL were improved. Hence, these cellular components may be useful as adjuvants for vaccines in aquaculture.

Key words: Oreochromis niloticus, vaccination, Lactobacillus plantarum VSG3, Hematology, immunity, biochemical parameters.

INTRODUCTION

The rapid development of aquaculture allied to intense Streptococcosis and lactococcosis, gram-positive bacteria, are major systemic bacterial diseases occurring in both wild and cultured fishes (Pridgeon and Klesius, 2012; Soltani *et al.,* 2005). During the examinations of the 108 gram-positive isolates cultured from diseased trout in seven provinces of Iran with major trout production from 2008 to 2009, 49 samples were identified as *S. iniae*, 37 samples were matched *Lactococcus garvieae*, and 22 samples identified as *Streptooccus* sp. (Haghighi Karsidani *et al.*, 2010), suggesting that the trout farms in Iran are severely affected by these diseases. Due to increasing in the resistant bacteria, demands for an effective vaccine have prompted over the past decade (Soltani *et al.,* 2007; Sun *et al.,* 2010; Pridgeon and Klesius, 2011 and Sun *et al., 2013*). Nonetheless, to date the initiative to control these diseases have focused on the use of antibiotics, including enrofloxacin, oxytetracycline erythromycin and amoxicillin (Austin and Austin, 2007 and Evans *et al.,* 2004). Currently, several experimental Streptococcosis and lactococcosis vaccines in the forms of formalin- killed (Soltani *et al.,* 2007), subunit vaccines (Cheng *et al.,* 2010; Zou *et al.,* 2011), DNA vaccines (Sun *et al.,* 2013), and attenuated live vaccines (Buchanan *et al.,* 2005) are reported. However, the only licensed vaccines against streptococcosis and lactococcosis are bacterins consisting of inactivated whole-cell bacteria (Sommerset *et al.,* 2005). In Iran, a bacterin vaccine is currently available to protect rainbow trout (*Oncorhynchus mykiss*) from streptococcosis/ lactococcosis, which has given impressive results (Soltani *et al.,* 2007). Bacterins have also been used to immunize farmed fish in Korea, Australia, Spain and Chile (Hastein *et al.,* 2005; Sommerset *et al.,* 2005 and Austin and Austin, 2007). However, the protectivity of these bacterins in some

cases was not completely satisfied (Bachrach *et al.*, 2001 and Eyngor *et al.*, 2008). Recently, the use of probiotic, especially lactic acid bacteria (LAB), as a preferable method that enhances the non-specific immune response of fish has been grown significantly. This improves the prevention and control of various diseases in aquaculture (Cruz *et al.*, 2012). *Lactobacillus plantarum* is a rod-shaped gram-positive bacterium belongs to LAB, and is known to produce plantaricin that is active against certain pathogens (Cebeci and Gurakan, 2003). In aquaculture, the administration of *L. plantarum* induced immune modulation, enhances the growth performance, and increases disease resistance in fishes (Son *et al*., 2009 and Giri *et al.*, 2013, 2014). Nonetheless, there is a lack of information regarding *L. plantarum* effects on vaccinated fish. Therefore, the present study was carried out to explore the influence of probiotic, *L. plantarum* on some serum biochemical and some immune parameters of vaccinated Nile tilapia.

Aim of this study is evaluated the effects doses of *Lactobacillus plantarum VSG3* at 10^4, 10^6, 10^8 and 10^{10} Colony Forming Units (CFU)/mL on the hematological, immunological IgM. Lysozyme activity and serum biochemical parameters on fresh water fish, Nile tilapia. So, the immunity and health of Nile tilapia treated with doses of *Lactobacillus plantarum VSG3* at 10^4, 10^6, 10^8 and 10^{10} Colony Forming Units (CFU)/mL were improved and can use for improvement of aquaculture.

MATERIALS AND METHODS

A commercial probiotic *Lactobacillus plantarum* VSG3 bought from the veterinary company was used in this study. The fresh water fish, Nile tilapia average weight 100gm $\pm$1gm were immunized intraperitoneally with 0.1 mL phosphate-buffer solution (PBS) containing *Lactobacillus plantarum VSG3* at 10^4, 10^6, 10^8 and 10^{10} Colony Forming Units (CFU)/mL were tested by intraperitoneal (i.p) injection. Fish were challenged 40 days after vaccination i.p. with doses of *Lactobacillus plantarum VSG3* at 10^4, 10^6, 10^8 and 10^{10} Colony Forming Units (CFU)/mL. Nile tilapia injected with 0.1 mL phosphate-buffer solution (PBS) served as the control. The Nile tilapia were acclimatized

for two weeks in the laboratory condition at Water quality parameters including water temperature, pH, dissolved oxygen and total ammonia were monitored daily and were maintained at 26±1.5ºC, 7.5±0.5, 8±0.4 mg/L and 0.55 ± 0.48 mg/L during the experimental period, respectively. and were fed a commercial diet 30% protein. The Nile tilapia were fed three times a day at rate of 3% of body weight. After checking the health status, acclimatized Nile tilapia were randomly distributed into 5 groups one control and four treated groups immunized intraperitoneally with 0.1 mL phosphate-buffer solution (PBS) containing Lactobacillus plantarum VSG3 at 10^4, 10^6, 10^8 and 10^{10} Colony Forming Units (CFU)/mL each in three replicate with 10 Nile tilapia per replicate. On 40 days of the experiment blood was collected from caudal vein. Then, serum biochemical and some immune parameters analysis were carried out on blood samples.

Hematological analysis:

At the end of the experiment, blood samples were collected from the Nile tilapia caudal vein by a sterile syringe containing heparin as an anticoagulant. Blood samples were placed into microtubes (2.0 mL) containing sodium heparin (50 IU) anticoagulants. All samples were collected in the early morning hours and were processed for hematological analysis. Samples were transported in a refrigerated cooler to the physiology Laboratory. Blood smears were prepared in duplicate and were stained with rapid hematological dye. The total cell count (erythrocytes, leukocytes, and thrombocytes) were performed by the diluent/dye direct method outlined by Natt and Herrick (1952) in a Neubauer chamber at a dilution of 1:100. Following the total cell count of nucleated cells (leukocytes and thrombocytes) in the Neubauer chamber, a differential count of leukocytes and thrombocytes were performed in the stained sample (Soivio & Oikari, 1976). The packed cell volume was determined by the microhematocrit technique described by Jain (1986). Blood was used for erythrocyte count (Dacie and Lewis 1984), hemoglobin content (Vankampen, 1961) and hematocrit value (Britton, 1963) determination. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were

calculated using the formulae mentioned by Dacie and Lewis (1984). Plasma was obtained by centrifugation at 3000rpm for 15min and the non-hemolyzed plasma was stored in a freezer at -20°C until analysis. Plasma protein content was determined by the Biuret method described by Wootton (1964). Glucose concentration was measured according to Trinder (1969), using Boehring Mannheium kits. Total lipids, cholesterol and triglycerides were determined calorimetrically using a kit supplied by El Nasr Pharmaceutical Chemical Co., according to Knight *et al*. (1972). Electrolytes, Creatinine, uric acid, alkaline phosphatase (ALP), Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined calorimetrically using kits supplied by Diamond Diagnostics, according to Reitman and Frankel (1975). Serum total IgM levels were measured with an Enzyme-linked Immunosorbent Assay (ELISA) using a commercial kit (Cusabio, Wuhan, Hubei, China), as described by Sun *et al.* (2010). Lysozyme activity was measured based on Ellis (1990) with a slight modifications carried out.

Statistical analysis:

The obtained data in this study were statistically analyzed for variance ANOVA, LSD (Least significant difference) according to (Snedecor and Cochran, 1982). Differences among treatment means were compared using Duncan's multiple range tests (Duncan, 1995). Data were presented as mean ± SE and significance was declared at ($P < 0.05$).

RESULTS

Hematology:

The number of red blood cells (RBCs), hemoglobin (Hb) and hematocrit (Hct) in Nile tilapia injected with *Lactobacillus plantarum VSG3* at 10^4, 10^6, 10^8 and 10^{10} Colony Forming Units (CFU)/mL were significantly higher than that of the control group after 40 days. Also, the number of Platelets, Mean cell (or corpuscular) volume (MCV), Mean cell hemoglobin (MCH) and Mean cell hemoglobin concentration (MCHC) of Nile tilapia injected with *Lactobacillus*

plantarum VSG3 were significantly higher than that of the control group after 40 days as shown table 1.

Table 1. Hematological changes due to effect of *Lactobacillus plantarum VSG3* at 10^4, 10^6, 10^8 and 10^{10} Colony Forming Units (CFU)/mL on Nile tilapia.

Doses / parameters	Control PBS	*L. plantarum VSG3* at 10^4	*L. plantarum VSG3* at 10^6	*L. plantarum VSG3* at 10^8	*L. plantarum VSG3* at 10^{10}
RBCs (x10^6/µL)	1.6±0.2	1.65±0.3	1.85±0.5*	1.95±0.7**	2.55±0.4**
HCT (%)	26.2±1.1	27.1±1.1	28.1±1.2 *	29.3±2.1 *	30.5±1.2**
Hb (g dL^{-1})	6.5±1.1	7.2±1.0	7.8±1.3*	8.0±1.2*	8.5±1.2**
Platelets 10^3mm^{-3}	277±1.7	280±1.8	298±1.7*	300±1.9**	321±1.1***
MCV fL10^{-15}	16.3±1.6	16.9± 2.6	17.5± 1.4*	17.9±1.4**	18.2± 2.1***
MCHC pg 10^{-12}	28.8±1.6	28.9±2.4*	29.5±1.3*	29.9±2.2*	30.6±1.1**
MCH g/dL	23.1±1.1	24.9±1.4*	25.1±1.1**	26.7±2.1***	28.1±1.1***

* Significant at p<0.05, ** highly significant at p< 0.01, *** very highly significant at p < 0.001 (significant differences between treated groups and control. Mean cell volume (MCV), Mean cell hemoglobin (MCH), Mean cell hemoglobin concentration (MCHC).

Serum biochemical parameters:

The results of serum biochemical parameters are presented in table 2. The alkaline phosphatase (ALP) levels of Nile tilapia injected with *Lactobacillus plantarum* VSG3 at 10^4, 10^6, 10^8 and 10^{10} Colony Forming Units (CFU)/mL were significantly higher than that of the control group after 40 days. While, showed significantly lower in levels of cholesterol, triglycerides, total lipids, HDL, LDL and glucose in the of Nile tilapia injected with *Lactobacillus plantarum* VSG3 than the control group. The total protein and IgM levels of vaccinated Nile tilapia with *L. plantarum* were significantly higher than those of the control group. However, no significant difference was found in albumin value in treatment groups compared to the control group. On the other hand, there are significant decreased in the serum levels of AST, ALT, uric acid creatinine and urea of Nile tilapia injected with *Lactobacillus plantarum* VSG3 at 10^4, 10^6, 10^8 and 10^{10} Colony Forming Units (CFU)/mL compared to control groups.

Table 2. Serum biochemical parameters changes due to effect of *Lactobacillus plantarum VSG3* at 10^4, 10^6, 10^8 and 10^{10} Colony Forming Units (CFU)/mL on Nile tilapia.

Doses / parameters	Control PBS	*L. plantarum VSG3* at 10^4	*L. plantarum VSG3* at 10^6	*L. plantarum VSG3* at 10^8	*L. plantarum VSG3* at 10^{10}
Glucose(mg/dl)	95 ±1.1	92 ±1.2*	90 ±1.6**	89±1.9**	77 ±1.5***
Total protein (g/dl)	6.2±0.2	6. 5 ± 0.1	6.9 ±0.5*	7±0.3*	7.9 ±0.5**
Albumin g/dl	1.4±0.1	1.44±0.1	1.46±0.2	1.5±0.1	1.6±0.2*
Total lipid	4.2±0.1	4.1±0.1	4±0.1*	3.3±0.1**	3.3±0.2**
Cholesterol(mg/dl)	221 ±11	196±16*	189±11***	183±16***	162±21***
Triglycerides(mg/dl)	3.2 ±0.3	3.1 ±0.1	3±0.1*	2.6±0.5*	2.2 ±0.6**
HDL CH mg/dl	3.1±0.2	2. 2 ± 0.1*	2.1 ±0.2*	2.0 ±0.2*	2.0 ±0.9**
LDL CH mg/dl	1.5±0.1	1.4±0.1*	1.3±0.2*	1.2±0.1**	1.1±0.2**
AST (u/l)	36 ± 1	32 ±4*	30 ±2**	26 ±0.56***	24 ±1***
ALT(u/l)	20±0.6	19±0.1	18 ±0. 7*	16 ±0. 2**	14.3±0.33***
ALP (u/l)	22±0.6	25±0.5	27 ±0.22**	28 ±0.3***	28±0.6***
Uric acid (mg/dl)	12 ±0.2	11 ±0.4*	10±0.1*	9±0. 3**	8±0.5***
Creatinine(mg/dl)	0.35 ±0.2	0.34 ±0.01*	0.31 ±0.02**	0.27±0.02**	0.24 ±0.03***
Urea (mg/L)	24±0.7	23±1*	22±1**	20±2**	18.1±4***

* Significant at $p<0.05$, ** highly significant at $p< 0.01$, *** very highly significant at $p < 0.001$ (significant differences between treated groups and control

Immune parameters:

Total leukocyte counts in immunized Nile tilapia injected with *Lactobacillus plantarum VSG3* were significantly higher than the control group. The level of lymphocytes and monocytes levels of fish injected with *Lactobacillus plantarum VSG3* and the lysozyme levels in Nile tilapia injected with *Lactobacillus plantarum VSG3* were significantly higher than the control group. Also, there are increased in the immunoglubin (IgM value) in the Nile tilapia injected with *Lactobacillus plantarum* VSG3 at 10^4, 10^6, 10^8 and 10^{10} Colony Forming Units (CFU)/mL for 40 days were significantly higher than the control group as shown in Table 3.

Table 3. Immune parameters changes due to effect of *Lactobacillus plantarum VSG3* at 10^4, 10^6, 10^8 and 10^{10} Colony Forming Units (CFU)/mL on Nile tilapia.

Doses/ parameters	Control PBS	*L. plantarum VSG3* at 10^4	*L. plantarum VSG3* at 10^6	*L. plantarum VSG3* at 10^8	*L. plantarum VSG3* at 10^{10}
Total Leuk. /µL	5456±122	5543±121*	5557±121*	5577±113**	5597±321***
Seg. Neutro. /µL	1655±111	1722±221*	1811±231**	1855±255***	1911±333***
Lymphocytes/µL	2133±112	2244±113*	2311±222***	2453±211***	2466±212***
Eosinophyls/µL	111 ±12	115±11*	146±11**	149 ±11***	151±12***
Basophyils/µL	211±23	215±21*	224±11**	226±31***	231±15***
Monocytes/µL	1400±211	1422±122*	1428 ±121*	1429±133**	1442±312***
Thromb/µL	34121±3311	34377±1545*	34499±4300*	34533±4433**	34555±1163**
IgM value (µg /ml)	22±0.7	23±0.8*	24±0.6**	25±0.9**	28±0.7***
Lysozyme (µg /ml)	13±0.7	16±0.9*	19±0.7**	21±1***	24±2***

* Significant at $p<0.05$, ** highly significant at $p< 0.01$, *** very highly significant at $p < 0.001$ (significant differences between treated groups and control.

DISCUSSION

The present study revealed that the total protein, lysozyme activity and IgM levels and leukocytes, lymphocytes, thrombocytes and red blood cells of vaccinated Nile tilapia injected with *Lactobacillus plantarum* VSG3 at 10^4, 10^6, 10^8 and 10^{10} Colony Forming Units (CFU)/mL for 40 days were significantly higher than those of the control group. However, no significant difference was found in albumin value in treatment groups compared to the control group. In contrast, the level of lymphocytes and monocytes levels of fish injected with *Lactobacillus plantarum* VSG3 at 10^4, 10^6, 10^8 and 10^{10} Colony Forming Units (CFU)/mL for 40 days. The lysozyme levels in Nile tilapia injected with *Lactobacillus plantarum* VSG3 at 10^4, 10^6, 10^8 and 10^{10} Colony Forming Units (CFU)/mL for 40 days was significantly higher than the control group. Also, there are increased in the immunoglubin (IgM value) in the Nile tilapia injected with *Lactobacillus plantarum* VSG3 at 10^4, 10^6, 10^8 and 10^{10} Colony Forming Units (CFU)/mL for 40 days were significantly higher than the control group. These results are agreement with Selim and Reda, 2015; Giri *et al.*, 2016 and Azza *et al.*, 2018. The probiotics have been recognized to function as immune-

modulators in finfish which is often through stimulation of innate and cellular immunity, including enhanced phagocytic, lysozyme, respiratory burst, cytotoxicity, complement activity, superoxide dismutase, increased numbers of leuko- cytes, erythrocytes, monocytes and lymphocytes, migration of neutrophils, neutrophil adherence, antiprotease and peroxidase activities, and plasma bactericidal activity (Newaj-Fyzul and Austin, 2015). In addition, there may be increases in serum bacterial agglutination antibody titer (Ridha and Azad, 2012), albumin (humoral immunity) and total IgM levels (Sharifuzzaman and Austin, 2010a, 2010b). Nonetheless, various probiotics may show different type of immune response. In the present study, serum biochemical parameters, including ALP, cholesterol, serum protein and total IgM values of vaccinated Nile tilapia the probiotic were significantly higher compared to the control group. The ALP is associated with the absorption of lipid, glucose, calcium and inorganic phosphate (Bailone, 2010; Giri *et al.,* 2013 and Meng *et al.,* 2018). In this study, the alkaline phosphatase (ALP) levels of all treatment groups were significantly higher than the control group. Increased phosphatase activity indicates a higher breakdown of the energy reserve, which may be utilized for the enhancement of growth or immunity (Meng *et al.,* 2018), as the result of vaccination and/or fed *L. plantarum* diet. Furthermore, the present study show the number (RBCs), (Hb) and (Hct) in Nile tilapia injected with *Lactobacillus plantarum* VSG3 at 10^4, 10^6, 10^8 and 10^{10} Colony Forming Units (CFU)/mL were significantly higher than that of the control group after 40 days. Also, the number of Platelets, (MCV), (MCH) and (MCHC) of Nile tilapia injected with *Lactobacillus plantarum* VSG3 at 10^4, 10^6, 10^8 and 10^{10} Colony Forming Units (CFU)/mL were significantly higher than that of the control group after 40 days as reported by (Bailone 2010 and Giri *et al.,* 2013.). Also, showed significantly lower in levels of cholesterol, triglycerides, total lipids, HDL, LDL and glucose in the of Nile tilapia injected with *Lactobacillus plantarum VSG3* at 10^4, 10^6, 10^8 and 10^{10} Colony Forming Units (CFU)/mL than the control group. The total protein and IgM levels of vaccinated with *L. plantarum* in the Nile tilapia were significantly higher than those of the control group. However, no significant difference was found in albumin value in treatment groups compared to the

control group. On the other hand, there are significant decreased in the serum levels of AST, ALT, uric acid creatinine and urea of Nile tilapia injected with *Lactobacillus plantarum* VSG3 at 10^4, 10^6, 10^8 and 10^{10} Colony Forming Units (CFU)/mL compared to control groups. This indicates the disorders of lipid and lipoprotein metabolism (Meng *et al.,* 2018). In the present study, the highest levels of serum IgM were observed in vaccinated Nile tilapia injected with *Lactobacillus plantarum VSG3* at 10^4, 10^6, 10^8 and 10^{10} Colony Forming Units (CFU)/mL. In spite of the slight decrease, its levels increased significantly more than the control group after 40 days of injection. Nonetheless, serum total IgM levels in unvaccinated Nile tilapia injected with *Lactobacillus plantarum* VSG3 at 10^4, 10^6, 10^8 and 10^{10} Colony Forming Units (CFU)/mL diet did not show a significant change compared to control group. These agreements with (Sun *et al.,* 2010), and in rainbow trout fed dietary *L. rhamnosus* (JCM 1136) up to 20 days (Panigrahi *et al.,* 2005) have shown an increase in IgM levels trend of feeding, and thereafter a dropping pattern prevailed. The total protein levels of vaccinated Nile tilapia injected with *Lactobacillus plantarum* VSG3 at 10^4, 10^6, 10^8 and 10^{10} Colony Forming Units (CFU)/mL were significantly higher than those of the control groups on all sampling days of the experiment. The increase in serum total protein content might be due to an increase in the leukocytes, which is a major source of serum protein production, including complement factors, lysozyme, and bactericidal peptides (Misra *et al.*, 2006). This is supported by increase in leukocytes value in the both vaccinated groups. Probably, the increase in the leukocyte count might have resulted in the enhancement of the non- specific immunity. Besides, the total leukocyte count, lymphocytes increased in both mentioned groups. Similar to pervious study by Soltani *et al.* (2007) who evaluated these parameters in immunized trout with streptococcosis/lactococosis vaccine. Significant higher serum lysozyme activity were shown in Nile tilapia injected with *Lactobacillus plantarum* VSG3 at 10^4, 10^6, 10^8 and 10^{10} Colony Forming Units (CFU)/mL compared to the control groups. These results has been previously showed in different studies (Son *et al.,* 2009 and Giri *et al.,* 2013). The discrepancy of these findings may

be attributable to the difference in probiotic doses and the feeding duration. Beside, vaccines have been shown to increase serum lysozyme activities, alternative complement activity and antibody titers as in rainbow trout (Kim and Austin, 2006 and Soltani *et al.*, 2007), which suggests an enhancement of these parameters due to using vaccine.

CONCLUSION

The present study can induce some of the specific and non-specific immune responses. This appears to be obtained by increasing lysozyme activity, immunoglubin IgM levels, hematological parameters and some serum biochemical parameters such as protein, ALP, Cholesterol, triglycerides, lipids, AST, ALT, uric acid, urea and creatinin levels. This study investigated a positive probiotic injected of Nile tilapia with *Lactobacillus plantarum VSG3* at 10^4, 10^6, 10^8 and 10^{10} Colony Forming Units (CFU)/mL in vaccinated Nile tilapia.

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تاثير العصيات النباتية المحسنة *Lactobacillus plantarum VSG3* علي تحسِّين مقاومة أسماك المياه العذبة للأمراض

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الملخـص العربـي

في هذه الدراسة، تم تقييم فعالية مناعية من المكونات الخلوية من البكتيريا البروبيوتيك في البلطي النيلي. تم تحصين البلطي النيلي معدل وزن ١٠٠ جم $\pm$ 1 جم في الغشاء البريتونى مع محلول فوسفات ١٠/١ ملم٣ (يحتوي على *Lactobacillus plantarum VSG3* عند 10^4 ، 10^6 ، 10^8 و 10^{10} وحدات تشكيل المستعمرات (CFU / mL) تم اختبارها عن طريق الحقن داخل الغشاء البريتونى. تم تحدي الأسماك بعد ٤٠ يومًا من التطعيم. مع جرعات من *Lactobacillus plantarum VSG3* عند 10^4 ، 106 ، 10^8 و 10^{10}وحدات تشكيل المستعمرة و الأسماك التي تم حقنها مع ١٠/١ ملم٣ محلول الفوسفات للمجموعة الضابطة. أوضحت النتائج أن إعطاء المكونات الخلوية يزيد بشكل كبير من نشاط الليزوزيم المصل والبروتين الكلي في الدم والجلوبيولين المناعي IgMابعد الفترة التجريبية. وأوضحت النتائج ان مستوى الجلوكوز والدهون والكوليسترول والدهون الثلاثية و ASTو ALTو ALP وحمض اليوريك واليوريا ومستويات الكرياتينين أقل من المجموعة الضابطة. أظهرت النتائج ان التطعيم بالبكتيريا المحسنة يزيد من الخلايا اللمفاوية بشكل ملحوظ في الأربع مجموعات المعالجة بالمقارنة بالمجموعة الضابطة. تشير هذه النتائج إلى أن المكونات الخلوية لبكتيريا الكائنات الحية المجهرية يمكنها التأثير على الاستجابات المناعية وتعزيز حماية الامراض وتحفيز التعبير الجيني المناعي في البلطي النيلي. وبالتالي، قد تكون هذه المكونات الخلوية مفيدة كمساعدات للقاحات في تربية الأحياء المائية. أظهرت النتائج ان التطعيم بالبكتيريا المحسنة يزيد من عدد كريات الدم الحمراء في المجموعات المعالجة اعلي من المجموعة الضابطة. بعد التحدي، كان العدد الإجمالي للصفيحات أعلى في الأسماك التي حصلت على أكبر جرعة من اللقاح. أظهر العدد الكلي للكريات الدم البيضاء وعدد الخلايا الليمفاوية أعلى القيم في الأسماك الملقحة. أظهرت النتائج ان التطعيم بالبكتيريا المحسنة انخفاض القيم

من الجلوكوز والدهون والكوليسترول، triglycrides، AST، ALT، ALP ، حمض اليوريك ، اليوريا والكرياتينين. لذلك، أظهرت النتائج ان التطعيم بالبكتيريا المحسنة ان الأسماك التي تتأثر بجرعات مختلفة من *Lactobacillus plantarum* أظهرت أعلى في مستوى من الصحة والنشاط من تلك المجموعة الضابطة. أظهرت هذه الدراسة أن الحقن لمدة ٤٠ يومًا بجرعات مختلفة من *Lactobacillus plantarum* VSG3. ادي الي تحسين مناعة وصحة البلطي النيلي المعالجة بجرعات من *Lactobacillus plantarum VSG3* من المجموعة الضابطة. لذلك يمكن ان نوصي باستخدام هذه الانواع من البكتيريا المحسنة في زيادة الاستزراع السمكي.