Pathological evaluation to the effect of some probiotics on the health and immune status of Nile Tilapia (*Oreochromis niloticus*)

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SUMMARY

The safety and efficiency of *Bacillus subtilis* and/or *Lactobacillus acidophilus*, as potential probiotics, were evaluated histopathologically and immunologically besides after challenge infection through an experiment using 960 Nile tilapia (*Oreochromis niloticus*) reared in aquaria. The experimented fish divided into 4 equal groups. Groups 1- 3 fed daily on a basal diet supplemented with probiotics, the 4th group served as a control and fed on basal diet only. All fish fed at a rate of 5% of the body weight for 1 and 2 months.

The feed conversion and specific growth rates showed no significant change after one month of application but a significant to non-significant increase were remarkable after 2 months of treatment. The survival rate was significantly increased in the fish given *B. subtilis* and *L. acidophilus* for one and two months after application. The histopathological studies revealed minimal pathological alterations, in the examined organs of Nile tilapia from different supplemented groups, but an obvious activation in the hematopoietic tissues and melanomacrophage centers were recognized. The serum bactericidal activity and the mortality after the challenge infections were varied with the type of probiotic bacteria used, type of pathogen tested and period of application but as a general observation, it was high in the group that given a mixture of *B. subtilis* and *L. acidophilus* and groups treated for 2 months.

Based on the following observations where, the tested bacteria proved to have a potential probiotic effect, enhancing the immunity and health status of the experimental fish. Such probiotics promoted the resistance against the bacterial infections. No remarkable pathological alterations were recognized in groups treated with single or mixed probiotic candidate. It could be concluded that, one month application was sufficient and a mixture of the two probiotics was superior. However, full commercial cost benefit analysis is recommended.

INTRODUCTION

The intensive rearing of fish species in aquaculture generates a potentially stressful environment to the fish, with the possible suppression of the immune system, rendering the fish more susceptible to different diseases (Austin and Austin, 1999). The routine use of antibiotics during fish culture to minimize the risk of disease is not advisable since it may adversely affect the indigenous microflora of juveniles or adult fish and may increase the risk of promoting antibiotic-resistant microorganisms (Alderman and Hastings, 1998). Thus, the use of probiotics, in the culture of aquatic organisms, is increasing with the demand for more environment-friendly aquaculture practices (Gatesoupe, 1999).

A probiotic is generally defined as a live microbial food supplement which improves the balance of the host animal’s intestinal flora (Fuller, 1989). However in aquaculture, probiotics can be administered either as a food supplement or as an additive to the water (Moriarty, 1998). Probiotics in aquaculture 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 (Balcázar et al., 2007); and enhancement of nutrition of host species through the production of supplemental digestive enzymes (Ziaei-Nejad et al., 2006).

The lactobacillus sp. and bacillus sp. (Meunpol et al., 2003) are most commonly used probiotics in both humans and animals to prevent or treat gastrointestinal disorders (Ouwehand et al., 2004); improves animal growth and mitigates the effects of stress factors (Lara- Flores et al., 2003) and enhancing resistance to pathogens by activating both cellular and humoral immune defenses (Rengpipat et al., 2000).

Probiotics are widely used in poultry and swine rearing farms but little has been done to incorporate them into aquaculture. Thus, the current study aimed to pathologically and immunologically evaluate the efficiency of Bacillus subtilis and/or Lactobacillus acidophilus as a potential probiotic in the culture of Nile tilapia (Oreochromis niloticus).
MATERIAL AND METHODS

Fish:
Nine hundred and sixty apparently healthy Nile tilapia (O. niloticus) (5 ± 1.3 g, each) of both sexes were collected from the World Fish Center, Abbassa, Egypt. The fingerlings were equally allocated in 32 fully prepared glass aquaria (each of 60 x 70 x 50 cm and contain 150 L of water). They were kept for 2 weeks under observation for acclimation. The water was renewed daily. Low-pressure electric air pumps provided aeration via air stones and dissolved oxygen (DO) levels was maintained at or near the saturation levels. Water temperature was 26±1°C throughout the trial.

Bacterial strains:
Bacillus subtilis (B. subtilis) (ATCC 6633) was obtained as lyophilized cells from SIGMA. Lactobacillus acidophilus (L. acidophilus) was kindly supplied as a reference strain from the Animal Health Research Institute, Dokki, Egypt. The pathogenic strains, Aeromonas hydrophila (A. hydrophila), Pseudomonas fluorescens (P. fluorescens) and Streptococcus iniae (Strept. iniae) were obtained, as reference strains, from the Fish Health Laboratory at The World-Fish Center, Abbassa, Egypt.

Feeding and challenge experiment:
Nine hundred and sixty Nile tilapia fingerlings were divided into four equal groups, each of 240 fish. Each group was subdivided into 8 equal replicates to determine the probiotic-protective effect against challenge. The basal diet was fed to all fish during the week of acclimation. The 1st group was fed on diet supplemented with L. acidophilus (0.5 x 10^7 bacteria g^-1) and B. subtilis (0.5 x 10^7 bacteria g^-1). The 2nd group was fed on diet supplemented with L. acidophilus (1 x 10^7 bacteria g^-1). The 3rd group was fed on diet incorporated with B. subtilis (1 x 10^7 bacteria g^-1). The 4th group was given basal diet without probiotics (control). The fish were daily fed at a rate of 5% of the body weight for 8 weeks. All the diets were prepared twice a week and stored at the refrigerator temperature (4 ºC). The weight of all fish in each aquarium was weekly measured and the feed ratios were adjusted accordingly. The survival rate and the growth performance, serum bactericidal activity and histopathology, in addition to mortality after the challenge tests were determined at the end of the 4th and 8th weeks of the experiment.

Parameters evaluated:
1. Growth performance:
The feed conversion, condition factor and specific growth rate
were determined. Feed conversion rate was calculated according to the following formula:

\[ FCR = \frac{wf - wi}{F} \times 100, \]

Where: \(wf\) = final weight of fish (g), \(wi\) = initial weight of fish (g) & \(F\) = amount of feed (g).

\[ CF = \frac{weight}{total\ length^3} \times 100, \]

\[ SGR = \frac{Ln wf - Ln wi}{Tf - Ti} \times 100 \]

Where: \(wf\) = final weight of fish (g).
\(wi\) = initial weight of fish (g).
\((Tf - Ti)\) = time between the final and initial weight (days).
\(Ln\) = Logarithm to the base.

2. Survival rate:
The fish were counted after 4 and 8 weeks from the start of the experiment to determine the survival percentage (Survival % = No. of fish counted / No. of stocked fish x 100).

3. Serum bactericidal activity (SBT):
Twenty fish were randomly collected from each treatment together with the control. The fish were anesthetized by immersion in water containing 0.1 ppm tricaine methane sulfonate (MS-222). Whole blood (0.5 ml) was collected from the caudal vein of each fish using syringes (1-ml). The blood samples were centrifuged at 3000 xg for 15 min and the supernatant serum was collected and stored at -20 °C in screw capped glass vials until used for the serum bactericidal test. Broth 24 h bacterial cultures of A. hydrophila, P. fluorescens and Strept. iniae were centrifuged, and the pellet was washed and suspended in phosphate buffered saline (PBS). The optical density of the suspension was adjusted to 0.5 at 546 nm. This bacterial suspension was serially diluted (1:10) with PBS five times. The serum bactericidal activity was determined by incubating 2 µl of the diluted bacterial suspension with 20 µl of the serum in a micro-vial for 1 h at 37 °C. Sterile phosphate buffer saline replaced the serum in the bacterial control group. The number of viable bacteria was determined by counting the colonies after culturing on trypticase soya agar plates for 24 hr at 37 °C (Rao et al., 2006).

4. Histopathological examinations:
The twenty randomly collected Nile tilapia from each group, during blood collection after 4 and 8 week of experiment, were used for the histopathological examinations. Specimens from the internal organs of the infected fish were fixed in 10% phosphate buffer formalin. The fixed specimens were processed routinely. Five micron thick paraffin sections were prepared and stained with he-
matoxyl and eosin (H & E) (Carleton 1976).

5. Challenge test:

One month after the start of the feeding experiments, 60 fish were collected from each of the 3 probiotics supplemented and control groups and divided into three sub-groups, each of 20 fish that was then re-distributed equally among 3 aquaria. Fish from the 1st, 2nd and 3rd subgroup were challenged I/P with 0.5 ml of fresh culture suspension containing $10^8$ bacteria ml$^{-1}$ of A. hydrophila, P. fluorescens and Strept. iniae, respectively. The same challenge tests were repeated 2 months later on another 60 fish from each of the 4 groups. The challenged fish were kept under observation for 15 days and the dead fish were used for bacterial re-isolation and the mortalities were recorded.

6. Statistical analysis:

Analysis of Variance (ANOVA) and Duncan’s multiple Range Test (Duncan, 1955) was used to determine the differences between treatments. The mean values were significant at the level of (P<0.05). Standard errors, of treatment-means, were estimated. All the statistics were carried out using Statistical Analysis Systems (SAS) program (SAS, 2005).

RESULTS

Growth performance and survival rates:

The feed conversion ratio (FCR) and specific growth rate (SGR), after one month of feeding trial, showed no significant change in all supplemented groups in comparison with the control. After two months of experiment the FCR decreased while SGR increased in all treated groups than the control in a significant to non-significant manner. The condition factor, after the two feeding periods, was significantly increased in all treated groups than the control. The survival, after the two feeding periods, was significantly increased in groups received mixture of two bacteria (B. subtilus, and L. acidophilus) in comparison with untreated control group, also other treated groups showed higher survival than the control (Table 1).

Serum bactericidal activity:

The serum bactericidal activities against A. hydrophila, P. fluorescens and Strept. iniae were lowest in the control group and highest in the group that received mixture of the two bacteria (B. subtilus, L. acidophilus), after one and two months of experiment. Moreover, the viable bacterial counts of A. hydrophila, P. fluorescens and Strept. iniae were lower in two months than that in one month of experiment and also in all probiot-
ics treated groups in comparison with untreated control group or bacterial control (without serum treated). In addition to that, the viable bacterial counts in the group, that received a mixture of the two bacteria (B. subtilis, L. acidophilus), were lower than group received either L. acidophilus or B. subtilus (Table 2).

**Histopathological examination:**
**Group 1 (B. subtilis & L. acidophilus supplementation):**

The Nile tilapia, of the group fed on basal diet incorporated with a mixture of B. subtilis & L. acidophilus, revealed no marked difference in the microscopic picture at 4 and 8 weeks of experiment, however mild congestion in the blood vessels of the gill arch and in the central venous sinus of the gill lamellae was noticed (Fig. 1). No remarkable pathological alterations recognized in the gill arch and lamellae. The liver revealed congestion and vacuolation of some hepatic cells with nuclear pyknosis in some pancreatic acinar cells (Fig. 2). The musculatures exhibited mild edema and focal hyaline degeneration (Fig. 3) with some mononuclear cells infiltration in the dermis especially after 2 months of experiment. The spleen exhibited activation of melanomacrophage centers and focal hyperplasia in the lymph follicles (Fig. 4). The intestine displayed mucinous degeneration in the epithelial lining with mononuclear leukocytic infiltrations in the lamina propria. Focal epithelial desquamation was seen (Fig. 5). The kidneys showed no remarkable pathological changes but vacuolation of some renal tubular epithelium was noticed. Focal hyperplasia in the renal hematopoietic tissue and melanomacrophages was evident and increased at 8 weeks (Fig. 6).

**Group 2 (L. acidophilus supplementation):**

The Nile tilapia, of the group fed on basal diet incorporated with L. acidophilus, showed no marked pathological alterations at 4 and 8 weeks of experiment but edema and congestion in the gill arch were seen. Mononuclear cells were infiltrated the tope and base of the primary lamellae especially at 8 weeks of experiment. The secondary lamellae showed focal epithelial hyperplasia (Fig. 7). The musculatures revealed edema and focal hyaline degeneration. The liver showed nuclear pyknosis and focal hyperplasia in the hepatocytes with marked activation of melanomacrophage centers (Fig. 8). The pancreatic cells showed more basophilic cytoplasm with pyknotic or karryolytic nuclei. The intestine showed focal necrosis and epithelial desquamation with mucinous degeneration in the lining...
epithelium. The lamina propria showed numerous mononuclear leukocytic infiltration especially at 8 weeks of experiment. The kidneys showed mild tubular nephrosis mainly vacuolar degeneration in the renal epithelium. Focal hyperplasia in the hematopoietic tissue was evident.

**Group 3 (B. subtilis supplementation):**

The Nile tilapia, of the group fed on basal diet incorporated with B. subtilis, exhibited no marked pathological alterations at 4 and 8 weeks of experiment, however, mild congestion in the blood vessels of the gill arch and lamellae was observed. No remarkable pathological alterations recognized in the gill arch and lamellae (Fig. 9). The liver revealed vacuolation of most hepatic cells with eosinophilic cytoplasm of the pancreatic acinar cells. The musculature exhibited no remarkable changes. The intestine showed focal mucinous degeneration in the epithelial lining with edema and mononuclear leukocytic infiltration in the lamina propria that increased by time of experiment. The kidneys showed minimal pathological changes mainly vacuolar degeneration of some renal tubular epithelium. Focal hyperplasia in the hematopoietic tissue was evident (Fig. 10).

**Group 4 (The control):**

The internal organs of the control group revealed no marked pathological alterations with normal tissue architecture and cellular details.

**Mortality after challenge infection:**

The Nile tilapia that challenged with pathogenic strain from each of A. hydrophila, P. fluorescens or Strept. iniae (0.5 ml of $10^8$ bacterial cell suspensions) revealed higher mortality in untreated control group than other groups supplemented with single or mixture of probiotics (B. subtilus & L. acidophilus) which showed lower mortality in two months than in one month of probiotics supplementation. The degree of significance in mortality after challenge infection between different groups and at the two periods of experiment was recorded in Table (3).
Table (1): Food conversion rate, condition factors, specific growth rate and survival of *O. niloticus* after feeding probiotics for 1 & 2 months (mean ± Standard error).

| Group/ Treatments | One month | Two months |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
|-------------------|-----------|------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
|                   | FCR       | CF         | SGR      | Survival | FCR       | CF         | SGR      | Survival |
| 1. *B. subtilis* & *L. acidophilus* | 1.56A ± 0.04 | 2.05A ± 0.06 | 2.04A ± 0.16 | 97.0A ± 1.77 | 1.69A ± 0.04 | 2.14A ± 0.05 | 2.44AB ± 0.12 | 94.0A ± 2.93 |
| 2. *L. acidophilus* | 1.58A ± 0.64 | 2.02A ± 0.05 | 2.48A ± 0.16 | 91.0B ± 1.67 | 1.71AB ± 0.04 | 2.11A ± 0.04 | 2.53A ± 0.12 | 84.3B ± 1.87 |
| 3. *B. subtilis* | 1.59A ± 0.05 | 1.99A ± 0.63 | 2.44A ± 0.15 | 88.0B ± 1.34 | 1.71A ± 0.03 | 2.08A ± 0.05 | 2.49B ± 0.11 | 79.0B ± 2.38 |
| 4. Control | 1.70A ± 0.07 | 1.63B ± 0.16 | 1.93A ± 0.21 | 84.0B ± 1.67 | 1.82A ± 0.05 | 1.75B ± 0.12 | 2.03B ± 0.17 | 76.0B ± 2.86 |

Columns of the same letter are not significantly different.

Table (2): Serum bactericidal activity of *O. niloticus* against pathogenic bacteria after feeding probiotics for 1 & 2 months, (bacterial count mean ± Standard error).

| Group/ Treatments | One month | Two months |          |          |          |          |          |          |          |          |          |          |
|-------------------|-----------|------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
|                   | A. hydrophila | P. fluorescens | Strept. iniae | A. hydrophila | P. fluorescens | Strept. iniae |
| 1. *B. subtilis* & *L. acidophilus* | 355Ba ± 11.31 | 360Ca ± 25.8 | 411Ba ± 15.84 | 230Bb ± 11.82 | 275Cb ± 34.33 | 317Ba ± 25.26 |
| 2. *L. acidophilus* | 402Ba ± 30.13 | 460Ba ± 21.9 | 409Ba ± 42.72 | 274Ba ± 38.56 | 381Bb ± 29.11 | 298Ba ± 33.79 |
| 3. *B. subtilis* | 358Ba ± 11.62 | 390BcBa ± 33.75 | 451Ba ± 21.07 | 254Ba ± 22.5 | 325Bb ± 41.37 | 379Ba ± 35.34 |
| 4. Control | 553Ba ± 45.16 | 577Ba ± 11.81 | 597Ba ± 11.05 | 505Ba ± 54.79 | 571Ba ± 34.15 | 571Ba ± 31.23 |

Upper case letter-superstscripts denote significant differences among treatments within the same pathogen/ period.
Lower case letters-superstscripts denote significant differences between the two periods within the same treatment/pathogen.
Table (3): Mortality percent among *O. niloticus* after challenge infections at the end of 1st and 2nd months of feeding on probiotic-supplemented diet.

<table>
<thead>
<tr>
<th>Group/ Treatments</th>
<th>One month (%)</th>
<th>Two months (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A. hydro- philia</td>
<td>P. fluo- rescens</td>
</tr>
<tr>
<td>1. <em>B. subtilis</em> &amp; L. acidophilus</td>
<td>44&lt;sup&gt;Aa&lt;/sup&gt; ±4.3</td>
<td>50&lt;sup&gt;Ca&lt;/sup&gt; ±3.54</td>
</tr>
<tr>
<td>2. <em>L. acidophilus</em></td>
<td>50&lt;sup&gt;Aa&lt;/sup&gt; ±2.24</td>
<td>63&lt;sup&gt;Ba&lt;/sup&gt; ±3.74</td>
</tr>
<tr>
<td>3. <em>B. subtilis</em></td>
<td>53&lt;sup&gt;Aa&lt;/sup&gt; ±5.51</td>
<td>54&lt;sup&gt;BCa&lt;/sup&gt; ±3.74</td>
</tr>
<tr>
<td>4. Control</td>
<td>58.75&lt;sup&gt;A&lt;/sup&gt; ±6.57</td>
<td>76.25&lt;sup&gt;A&lt;/sup&gt; ±3.15</td>
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Upper case letter-superscripts denote significant differences between treatments within the same pathogen/ period. Lower case letters-superscripts denote significant differences between the two periods with the same treatment pathogen.
Figs 1- 6: Nile tilapia fed on diet incorporated with *B. subtilis* & *L. acidophilus*:
1. Gill showing congestion in the gill arch. H&E stain, x 250., 2. Liver showing congestion and vacuolation of the hepatic cells. H&E stain, x 100., 3. Muscles, at 8 weeks of experiment, showing mild edema and focal hyaline degeneration. H&E stain, x 250., 4. Spleen showing activation of melanomacrophage centers and focal hyperplasia in the lymph follicles. H&E stain, x 100., 5. Intestine showing mucinous degeneration and focal epithelial desquamation in the epithelial lining with mononuclear leukocytic infiltration in the lamina propria. H&E stain, x 250., 6. Kidneys, at 8 weeks, showing vacuolation of some renal tubular epithelium and focal hyperplasia in the hematopoietic tissue and melanomacrophages. H&E stain, x 100.
Figs 7-8: Nile tilapia fed on diet incorporated with *L. acidophilus*:
7. Gills, at 8 weeks of experiment, showing hyperplasia in the secondary lamellae. H&E stain, x 100.
8. Liver showed nuclear pyknosis and vacuolation in the hepatocytes with marked activation of melanomacrophage centers. H&E stain, x 100.

Figs 9-10: Nile tilapia fed on diet incorporated with *B. subtilis*:
9. Gills showing mild congestion and no remarkable pathological alterations in the gill lamellae. H&E stain, x 100.
10. Kidney showing mild tubular nephrosis with focal hyperplasia in the hematopoietic tissue. H&E stain, x 250.
DISCUSSION

The use of probiotic in aquafeeds has received considerable attention in recent years (Gatesoupe 1999; Verschuere et al. 2000). The rationale of their use in aquaculture is to improve feed intake and feed efficiency, survival and to minimize infection.

The obtained results showed that, the feed conversion ratio (FCR) and specific growth rate (SGR) were improved in the Oreochromis niloticus fingerlings that feed with probiotic–supplemented diets than those fed with the control diet particularly after 2 month. This may attributed to increases in specific activities of digestive enzymes in probiotic treatments (Ziaei-Nejad et al., 2006) that may have led to enhanced digestion and increased absorption of food, which in turn lead to the improved growth in fish. Our result in accordance with Lara-Flores et al. (2003), who reported that, the fry of Oreochromis niloticus fed on diets with a probiotics supplement exhibited greater growth than those fed with the control diet. In contrast, Shariff et al. (2001) and McIntosh et al. (2000) found that, the treatment of P. monodon and Litopenaeus vannamei with a commercial Bacillus probiotic did not significantly increase growth. The variation in result may attributed to the type of bacteria used, duration of exposure, and state (live or dead) of bacteria (Gomez –Gil et al., 1998).

The used probiotic significantly improved fish survival in most treatments because probiotic are able to out-compete other bacteria for nutrients and space and can exclude other bacteria through the production of antibiotics (Moriarty, 1998; Verschuere et al., 2000). The administration of both probiotics have been shown to increase fish survival by enhancing resistance to pathogens through activating both cellular and humeral immune response. This finding was confirmed by our histopatological findings where focal hyperplasia in the hematopoietic tissue was evident and also immunologically through the recorded increase in the serum bactericidal activity of O. niloticus against pathogenic bacteria. These results are in accordance with Panigrahi et al. (2007) and Huang et al. (2008).

The increase in serum bactericidal activity of Oreochromis niloticus against pathogenic bacteria in comparison to the control especially after 2 months may attributed to either the antimicrobial substances that produced by L. acidophilus and B. subtilis (Smorgiewicz et al. 1993) or to the increased natural complement, serum peroxidase and phagocytic ac-
tivities (Salinas et al., 2008). These findings were in agreement with Paturi et al., (2008) who reported that, the phagocytic activity of the peritoneal macrophages was significantly higher in mice fed either L. acidophilus or L. paracasei compared with control mice. The serum bactericidal activity was significantly higher in the group received a mixture of probiotics compared to those supplemented with single probiotic species or the control groups, this observation was in agreement with Salinas et al., (2008).

The microscopic examination, in the current study, revealed minimal pathological alterations in different supplemented groups with no remarkable difference between either groups or period of experiment. The histopathological findings, among all supplemented groups at the two periods of experiment, summarized a mild to moderate circulatory disturbances, mainly congestion was seen in the gill arch and lamellae. Focal degenerative changes, mainly vacuolar degeneration in the hepatocytes and renal epithelium besides mucinous degeneration in the intestinal epithelium were noticed. This in addition to, mononuclear cell infiltration in the gill lamellae, dermis and intestinal lamina propria besides hyperplasia in the hematopoietic tissue were apparent. Our histopathological results are in agreement with Babińska et al., (2005) who reported no negative impact of L. acidophilus on the morphology of the liver and gastrointestinal tract when given in piglets feed. The activation of the hematopoietic tissue, in the present study, was in accordance with Sato et al. (1984).

The mortality, in the current study, among O. niloticus that challenged by each pathogen (A. hydrophila, P. fluorescens or Strept. iniae) at the end of 1st and 2nd months of feeding on probiotic-supplemented diet was lower than the control group. The obtained results were in accordance with the result reported by Cano and Perdigon (2003); Balcázar et al., (2007) and Truusalu et al., (2008). The low mortality might attributed to the substances produced by L. acidophilus and B. subtilis like antimicrobials, lactic and non-lactic acids, hydrogen peroxide which inhibit or kill pathogens (Servin 2004). Moreover, L. acidophilus and B. subtilis in the gut compete with the pathogen for the adhesion sites and nutritional sources (Smoragiewicz et al., 1993; Marteau et al., 2001). This in addition to the immune-modulation of the host that either increase the resistance against pathogens (Rengpipat et al., 2000) or inhibit the production of
bacterial toxins (Alakomi et al., 2000).

Conclusion:

The tested bacteria showed a potential probiotic effect and promoted the resistance against the bacterial infections without remarkable pathological alterations that indicate the safety of the selected isolates as a probiotics. A mixture of the two probiotics was better than single and economically one month application was effective.

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تقييم باثولوجيجي لتؤثير بعض البر ويبوتوك على الحالة الصحية والمناعية لأسماء البطة النيلية

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المخصص العربي

تم تقييم هستوباثولوجيجي ومناعي لمدى أمان وكفاءة كل من الباسيلس ستيلس واللاكتوباسيلس أسينوفيلس كرويونυςك ويبوتوك، وبعد إجراء العدوى التجريبية خلال تجربة استخدم فيها 90 من أسماء البطة النيلية التي ربت في أحواض زجاجية وتغذت على غذاء متكامل بنسبة 5% من وزن الأسماك المجربة لمدة شهر وشدرين.

ولم يظهر تغيير معنوي في معدلات التحويل الغذائي والنمو بعد شهر من استخدام هذه البكتيريا بينما ظهرت فروق معنوية بعد شهرين. وقد أرادة نسبة الإعاقة معنوية في الأسماك المجررة بكل من الباسيلس ستيلس واللاكتوباسيلس أسينوفيلس بعد شهر وشدرين من المعاينة. وقد أسفرت الاختبارات الهستوباثولوجية عن تغيرات طفيفة في الأعضاء المفتوحة من الأسماك المجررة في جميع المعاينة بينما اضح تناقل في الأسماة المناعية ومراكز الميلانومكروفاج. وقد تباعضت نتائج اختبار النشاط القاتل للبكتيريا وكذلك نسبة النفوذ بعد العدوى التجريبية باختلاف نوع البر ويبوتوك المستخدم وكذلك نوع الميكروبي الضار المختبر وفترة الاستخدام ولكن بصفة عامة تبين ارتفاع قيم النتائج في المجموعة المجررة بزيادة من كل من الباسيلس ستيلس واللاكتوباسيلس أسينوفيلس وكذلك في المجموعات المجررة لمدة شهرين.

وقد تم تحديد كفاءة البكتيريا المختبرة كبر ويبوتوك حيث أنها أحدثت نشاطاً في الجهاز المناعي والحالة الصحية للأسماء المجررة. كما ساعدت على مقاومة الأسماك للعدوى التجريبية. ولم يظهر تغييرات معنوية على أسماك المجموعات المجررة بأي من البر ويبوتوك المستخدمة على حدة أو بصورة مختلطة. وخلص البحث إلى استبان كفاءة إضافة هذه البر ويبوتوك لمدة شهرين وكذلك على صورة مختلطة ومع ذلك يوصي بعمل تقييم اقتصادي لتلك المعاينة قبل التوصية باستخدامها على نطاق واسع في مجال الاستزراع السمكي.

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