EFFECTS OF DIETARY COMPOUND PROBIOTICS ON THE GROWTH PERFORMANCE AND INNATE IMMUNE RESPONSE OF NILE TILAPIA

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Received: 30 December 2018; Accepted: 30 January 2019

ABSTRACT

This study was conducted to investigate the effect of addition of two commercial compound probiotics to the Nile tilapia fish’s diets on growth performance and distributed into 3 groups (control and 2 experimental) 30 fish each in three replicates. Fish were mentioned for adaptation in running water for 21 days. Duration of the experimental period was extended for 30 days. Fish were weighted at the beginning and at the end of the experiment. Blood samples were collected for determination of the immune and biochemical parameters, the results indicated a positive effect represented by significant increase in RBCs count, PCV%, Hb Conc., WBCs and differential leukocytic count. The current study clearly demonstrated that compound probiotics can be used to modulate the immune response of Nile tilapia and to enhance the growth performance of Nile tilapia fish.

Key words: Nile tilapia; Compound Probiotics; Growth performance; Blood biochemistry; Non-specific immunity.

INTRODUCTION

Actually, with the growing human population, aquacultures in Egypt are growing fastest to compensate the shortage in animal protein. Among the cultured species was Nile tilapia, O. niloticus which is the potential and predominant cultured species around the world and comprises over 70% of the cultured tilapia (Fitzsimmons, 2000 and Fitzsimmons, 2004).

Several disease problems are accompanied by rapid growing and intensification of aquaculture, often due to opportunistic pathogens. Increased stocking densities with increased feeding rates and other organic loads enhances proliferation of opportunistic bacteria (Austin et al., 1995) inflicting adverse effects on fish health and production efficiency with consequent mass mortality.

Though the common response to prevention and control of diseases through the application of antibiotics and chemotherapy, the wide spread use of chemotherapeutics, especially antibiotics has resulted in emergency of drug resistant bacteria (Smith et al., 1994), accumulation of residual antibiotics in fish tissues (Harikrishnan et al., 2009a and Harikrishnan et al., 2009b) and environmental pollution

(Smith et al., 1994). Recently, in aquaculture, the use of probiotic bacteria to enhance the growth performance has received considerable attention. Using probiotics in aquaculture have been reported to provide beneficial effects (Balcázar et al., 2006) and the use of probiotics for enhancing bio-growth parameters has been well documented in fish (Robertson et al., 2000).

Numerous studies have shown that the addition of probiotics can improve feed conversion, growth rates and weight gain of salmonids (Merrifield et al., 2010). Probiotics are important for weight gain in fish (Aly et al., 2008, Cheng et al., 2014 and Dawood et al., 2015).

In tilapia, a number of commercial probiotics consisting of one or a mixture of bacteria such as Bacillus, Streptococcus, Lactobacillus or the commercial yeast Saccharomyces were used and reported higher growth performance and better immune response than the untreated fish (El-Haroun et al., 2006). Feed supplemented with multi-species probiotics is probably more effective than mono-species probiotics in provision of more diversity in antimicrobial component (Nayak, 2010a).

The use of multi-species probiotics as feed complements is probably more influential than mono-species probiotics in terms of more diversity in antimicrobial compounds and higher adhesion on the gut mucus (Nayak, 2010a). It has recently been shown that a mixture of Bacillus probiotics has
significantly increased growth rate and body weight in the sea bream Sparus aurata (Avella et al., 2010). Generally, probiotics are mostly studied separately. Limited studies have contemplated the use of two or more bacteria as a probiotic mixture in fish (Nayak, 2010c and Dimitroglou et al., 2011). Two studies, of (Salinas et al., 2005 and Salinas et al., 2008) indicate that the two different bacteria could be more efficient and more proportionate than a single strain probiotic. Moreover, a combination of four bacteria was more beneficial to rainbow trout (Oncorhynchus mykiss) (Irianto and Austin, 2002). So, mixed cultures may contain bacteria that complement each other’s health effect and thus differently modulate host immune responses (Dimitroglou et al., 2011).

Recently, lactic acid bacteria (LAB) probiotics as a feed supplement have been shown to be effective to modulate immunity and resistance against infectious diseases (Merrifield et al., 2010; Nayak 2010 b; Nayak 2010c and Dimitroglou et al., 2011).

Dietary administration of Lactobacillus spp. modulate the non-specific immune responses, growth and disease resistance of Nile tilapia (O. niloticus) (Ngamkala et al., 2010).

The innate immune system of fish is very important which enables a rapid response against invading pathogens, and intensification of culture practices requires the use of external feed additives (Essa and Salama, 1994) to improve the growth performance and the immune response (Merrifield et al., 2010, Nayak 2010 b, Nayak 2010 c and Dimitroglou et al., 2011). Therefore, greater emphasis on application of probiotics in aquaculture is a better alternative to the drawbacks of using antibiotics (Watson et al., 2008).

The effect of probiotic on blood parameters have been studied in a number of studies (Merrifield et al., 2010). Hematological techniques have proved to be valuable for assessing the health of fish and monitoring stress responses. There were a range of factor that affect the hematological parameters of fish, among them the dietary regime used (Osuigwe et al., 2005).

The present experiment was conducted to evaluate the effects of addition two commercially available probiotic products, (Pro-bac plus and Amphi-bac), to Nile tilapia diets on the growth performance and some hemat- biochemical immune parameters.

**MATERIALS AND METHODS:**

**Fish:**
Nile tilapia, O. niloticus (n=90) were collected from a private fish farm in Qena province, Egypt. Fish were transported to the laboratory of Fish Diseases Department at Faculty of Veterinary Medicine, South Valley University, Qena, Egypt. Fish were maintained under laboratory circumstances for adaptation in running water for 21 days depending on the protocol of maintaining bioassay fish as was previously described by Ellsaesser and Clem (1986) and kept in 9 plastic aquariums at density of 10 fish per aquarium. Fish were fed with basic diet during acclimation until the beginning of the experiment. Water change rate was seventy percent daily with dechlorinated stagnant tap water. Dissolved oxygen level was maintained by aeration above 5 mg/L.

**Experimental diet:**
The basic diet was formulated physically to contain about grounded yellow corn 34.9%, vegetable bean meal 28.6%, fish meal 17.0%, wheat bran 9.3%, mineral mixture 1.7% and vitamin mixture 1.0%.

The basal diet was used as the control diet (diet 1). Two experimental diets (diet 2 & 3) were formulated from the basal diet. Apart of the basal diet was supplemented with commercial compound porobiotic at the rate of 5gm/kg to form diet 2&3.

**Probiotic product added to diet 2 contain**
- LAB culture 2.0x10⁷ CFU/gm total bacteria (Lactobacillus acidophilus, L. Planterm, L. Bervis and Bifidobacteria).
- Enzymes blend concentrate, Amylase 3.45 units/gm.
- Beta-glucanase.
- Hemicellulase.
- Saccharomyces cervisiae 100mg/gm.

The second commercially available probiotic products (diet 3) contains:
- LAB culture 5.0 g/kg, provide a total of NLT 2.0x10⁹CFU/gm total bacteria (50% Lactobacillus acidophilus 2.50 gm/kg, 49% L. planterm2.45 gm/kg and 1% Bifidobifidum 0.05 gm/kg).
- Bacillus subtilis fermentation extracts 1.00 gm/kg.
- Aspergillusniger fermentation extract 1.00 gm/kg.
- Enzyme blend concentrate 2.00 gm/kg, provide a total enzyme of 34.5 units/gm (37% Amylase 1.46gm/kg, 12% Cellulase 0.24gm/kg, 6% Beta-glucanase 0.12gm/kg and 9% Hemicellulase 0.18gm/kg).
- Dextrose 993gm/kg.

The dietary ingredients were thoroughly mixed and stored at ~5 °C until used and the required amount of the diet was prepared every week.

**Experimental design:**
Fish (n=90) were divided into 3 main groups, each group was divided into 3 replicates (10 fishes each):
- The first group was control (n=30) fed on basic diet (diet 1),
- The second group (n=30) fed on basic diet supplemented with 5 grams probiotic/ kg diet (diet2)
- The third group (n=30) fed on basic diet supplemented with 5 gm probiotic/ kg basal diet (diet 3).

Each group was fed twice daily at 3% of body weight for 30 successive days. The weight of the fish was recorded at the beginning and at 30-day of the experiment.

**Growth parameters:**

Body weight development:
Live body weight of fish was recorded at the beginning of the experiment and at the end of the experiment (30 days)

Body weight Gain:
- BWG of fish was calculated by subtracting the live body weight at the beginning of the experiment (initial weight) from that at the end of the experiment initial weight of fish under experiment. (30days).
- BWG = Final weight - Initial weight

**Hematological studies:**
At the end the experiment (30 days), the weight of the fish was recorded and blood samples were collected from the caudal vein and divided into 2 portions. Sodium citrate (3%) was added to one part for evaluation of Hemoglobin concentration, packed cell volume, differential leukocytic count and phagocytic assay.

The second part of blood sample was allowed to clot, centrifuged at 3000 rpm for 15 min. for serum separation.

Hemoglobin was assessed by a spectrophotometer (unico UV-2150) at 540 nm through the method cyanmethemoglobin, Hematocrit was measured by the procedure microcentrifuge with heparinated tubes (Svetina et al., 2002). Differential recognition of the WBC was based on blood films preparation and Gimsa staining. Blood cell indices (MCH, MCV, and MCHC) were assessed according to the standard calculations described previously by (Campbell and Ellia, 2007).

Total serum proteins (g/dl) and albumin (g/dl) were determined by Protein and Albumin kits using colorimetric method according to manufacturer's recommendation. Globulin level (g/dl) was calculated mathematically by subtracting albumin value from total protein value (Busher, 1990).

**Phagocytosis assay:**
Phagocytic assay was conducted as per (Kawahara et al., 1991). *Candida albicans* culture 50μg was added to 1ml of citrated blood and shaken in water bath at 25°C for 5 hours. After that, smears of the blood were stained with Giemsa stain. Phagocytosis was evaluated by determining the proportion of phagocytic cells which contained intracellular yeast cells in a random count of 200 phagocytes (Abu-Elala et al., 2013) and expressed as percentage of phagocytic activity (PA). The phagocytic index (PI) was estimated by counting the number of phagocytized yeast cells in the phagocytic cells.

**Statistical analysis:**
All data were analyzed by one-way analysis of variance (ANOVA) using the general linear models procedure of statistical analysis system (SAS) version 8.02. Duncan's multiple range test (Duncan, 1955) was used to resolve differences among treatment means at 5% significant level.

**RESULTS**

Results of the blood immune parameters and weight gain of Nile tilapia (*Oreochromis niloticus*) which fed diets supplemented with Pro-bac plus or Amphi-bac are shown in (Table 1). Significant higher level of PCV and hemoglobin concentrations were recorded in fish fed on diets supplemented with probiotic products (diet 2&3) compared with the control.

Significant higher level of lymphocytes in fish received diet 3 probiotic than the control group, while neutrophil showed significant lower level than the other two groups.

Significant maximum phagocytic activity of Nile tilapia phagocytes was observed in fish received the diet 3 compound probiotic than the other two groups, while the phagocytic index was not significantly different for any diet group.

Significant higher values of total protein and globulin were recorded in fish groups received either type of probiotic diets, than that of control group, while albumin level showed significant decrease.

Fish in the second and third groups fed on diets containing probiotics products recorded significantly higher BWG than the control one.
DISCUSSION

Hematological data of the current study showed that HB and PCV were affected by probiotics used. Also (Marzouk et al., 2008a) found increases in HB value and PCV in fish groups fed with diet supplemented with probiotic (B. subtilis and Saccharomyces cerevisiae). The increase in blood parameters could be attributed to the fact that, application of probiotics leads to hemopoietic stimulation.

Obtained results recorded that white blood cell count was increased in fish of the experimental groups in comparison with the control and this was an inevitable result because probiotics effect on immune system which might appear in white blood cell density (Panigrahi et al., 2005) and may increase phagocytosis (Picchietti et al., 2007).

The innate immune system of fish is the first line and primitive of defense against invading pathogens. The major components of the immune system are macrophages, monocytes, granulocytes, and humoral elements, such as immunoglobulins (Secombes and Fletcher, 1992 and Magnadottir, 2006). Several types of leukocytes participate in the cellular immune response, including lymphocytes, monocytes, granulocytes (neutrophils, eosinophils and basophils), and cytotoxic cells (Nakanishi et al., 1999). Concerning the non-specific immune stimulation in O. niloticus fish groups received diets supplemented with probiotics. It was clear that high non-specific immunity was developed as manifested by increased number of lymphocytes and monocytes in the differential leucocytic count due to the activation of the hemopoietic tissues. In addition, recent studies indicated that probiotics can stimulate the piscine gut associated lymphoid tissue provoking an immune response (Nayak 2010a).

In the present study, better concentrations of Hb, % haematocrit, WBC, total serum protein, and serum globulin were observed in Nile tilapia maintained on the diet supplemented the two commercial diet used in the experiment, showing significant differences (P<0.05) from the control. All leucocytes were counted as a percentage of the whole leucocytic count which constitutes 100 %, so either increase or decrease in different leucocytes was pronounced in fish. The decrease of the percentage of neutrophils in fish groups fed on Amphi-bac diet may be attributed to the significant increase of other leucocytic cells especially lymphocytes. Phagocytosis is a primary, non-specific defense mechanism against invasion of pathogenic organisms of hosts (Olivier et al., 1985). In the current study, Nile tilapia phagocytes showed increased the phagocytic activities when fed on either Pro-bac or Amphi-bac diets. The enhancement of phagocyte function is one of the most immediate and key effects produced by probiotics on the host immune system of fish. Previous studies demonstrated that aquatic animals fed probiotic had significantly increased phagocytic activity and index (Dias et al., 2010 and Harikrishnan et al. 2010). Increase in the percent of phagocytosis could be attributed to that probiotics used activate the phagocytic cells in the hemopoietic organs. The results indicated a significant increase in total protein and globulin in the second and third groups compared with the control group fed the basic diet, which could be attributed to the immuno- modulatory effect of Probac and Amphibac diets on the liver cells which activate the anabolic capacity of the hepatocytes to produce blood proteins particularly globulin. The present findings confirmed by those of Marzouk et al.

Table 1: Immune parameters and body weight gain of Nile tilapia as influenced by the different dietary treatments.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Basic diet</th>
<th>Pro-bac diet</th>
<th>Amphi-bac diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin gm/100ml</td>
<td>2.5±0.1 c</td>
<td>3.6±0.1 b</td>
<td>4.0±0.2 a</td>
</tr>
<tr>
<td>PCV%</td>
<td>24.5±0.7 c</td>
<td>30.8±0.7 b</td>
<td>34.2±1.8 a</td>
</tr>
<tr>
<td>Lymphocyte %</td>
<td>41.4±3.4 b</td>
<td>42.2±1.8 a</td>
<td>47.8±1.5 a</td>
</tr>
<tr>
<td>Monocyte %</td>
<td>27.4±2.3 a</td>
<td>31.6±2.1 a</td>
<td>31.8±2.2 a</td>
</tr>
<tr>
<td>Neutrophil %</td>
<td>18.2±1.2 a</td>
<td>15.4±1.8 a</td>
<td>13.6±1.8 b</td>
</tr>
<tr>
<td>Basophile %</td>
<td>8.2±0.9 a</td>
<td>7.6±0.97 a</td>
<td>5.8±1.2 a</td>
</tr>
<tr>
<td>Eosinophil %</td>
<td>4.8±0.5 a b</td>
<td>3.2±0.7 a</td>
<td>1±0.3 b</td>
</tr>
<tr>
<td>Phagocytic %</td>
<td>21.7±0.98 b</td>
<td>24.1±1.2 b</td>
<td>28.2±1.4 a</td>
</tr>
<tr>
<td>Phagocytic index</td>
<td>1.06±0.03 a</td>
<td>1.10±0.03 a</td>
<td>1.13±0.03 a</td>
</tr>
<tr>
<td>Total protein mg/dl</td>
<td>3.32±0.1 b</td>
<td>4.46±0.2 a</td>
<td>4.1±0.2 a</td>
</tr>
<tr>
<td>Albumin mg/dl</td>
<td>1.8±0.1 a</td>
<td>1.4±0.2 b</td>
<td>1.26±0.1 b</td>
</tr>
<tr>
<td>Globulin mg/dl</td>
<td>1.5±0.2 b</td>
<td>3.06±0.3 a</td>
<td>2.84±0.1 a</td>
</tr>
<tr>
<td>Total weight gain /gm</td>
<td>5.4±1.4 b</td>
<td>10.7±1.6 a</td>
<td>11.8±1.5 a</td>
</tr>
</tbody>
</table>

Means in the same raw with different letters are significantly different (p<0.05).
(2008b); Zhou et al. (2010) and Chelladurai et al. (2013) who reported that total protein level was significantly increased by using probiotics and these results were supported by several authors Ortuño et al. (2002); and Safinaz (2006). With regard to the increase of the total protein and albumin in the treatment group, it can be concluded that the usage of the increase of the mentioned factors. In the confirmation of the above findings.

Significant increase in globulin in Pro-bac and Amphi-bac diets groups consequently lead to significant decrease in albumin, as globulin obtained by direct subtracting the values of the albumin from those of the total protein. Our results concurred with speculations of other previous studies with regard to the total immunoglobulin concentration, where it was concluded that fish immunoglobulin concentration increases with probiotic in the diet. Increased total immunoglobulin concentration could be due to an increased immune response in the probiotic groups, induced by the presence of L. acidophilus, as suggested by Panigrahia et al. (2005).

Recently, it became known in aquaculture that probiotics in diets could help to improve fish growth. Among the various benefits of probiotics in aquaculture is the increased growth rate of fish (Ghosh et al., 2008 and Merrifield et al., 2009) and this could be confirmed in our experiment. This benefit is supposed to occur via the bacterial species colonizing the gut of the host as probiotics lead to a change in the bacterial composition of the gut that in some way benefits the health of the host (Balcázar et al., 2006 and El-Haroun et al., 2006).

In the present study, significantly (P<0.05) better growth performance was observed in Nile tilapia maintained on the diet supplemented with the two commercial probiotics compared with fish of the control group fed on the basal diet. Ramesh et al. (2015) suggested that probiotics have beneficial effect on digestive operation because probiotic strains synthesize extracellular enzymes such as proteases, amylases and lipases, therefore, nutrients are absorbed more efficiently when the feed was supplemented with probiotics. Similar to our results, Lara-Flores et al. (2003) found that using yeast and the microbial mixture as feed supplement led to the best growth than those of control. Also Mohamed et al. (2007) reported that feeding diets supplemented by probiotic to fed Nile tilapia (O. niloticus) fingerlings exhibited greater growth than those of control.

Improvement in growth performance when a commercial probiotics were used in fish diet, could be due to better nutrient digestibility, high-quality absorption and increased enzyme activities caused by a proper balance of the intestinal microbial flora (Fuller, 1989) or exoenzyme secretion as suggested by Yanbo and Zirong (2006).

**CONCLUSION**

The present experiment showed that compound probiotic as feed supplement can be considered as an alternative method to overcome the drawbacks of chemotherapeutics for health improvement in aquaculture.

Results of the current study proved that dietary supplementation of Pro-bac or Amphi-bac diets enhanced the growth performance and the overall immune response of Nile tilapia as was indicated by the significant increase of the hematologic parameters, globulin proteins and the phagocytic activities of fish phagocytes.

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Duncan, D.B. (1955): Multiple Range and Multiple


Tahir et al. (2010) investigated the effect of probiotic supplementation on the growth and immune response of Nile tilapia (Oreochromis niloticus). The study found a significant increase in the number of white blood cells in the groups fed with probiotics compared to the control group. The results also showed a positive effect, evidenced by a significant increase in the number of red blood cells, PCV%, hemoglobin concentration, and the number of white blood cells. Additionally, there was an increase in the percentage of phagocytosis by yeast cells in both groups fed with probiotics compared to the control group. Moreover, there was a noticeable increase in the weight of the fish fed on the diet supplemented with probiotics, compared to the control group. The study concluded that probiotic supplementation can be used to improve the immune response of Nile tilapia and enhance growth performance.