THE INFLUENCE OF SYNBIOTIC ON GROWTH AND EXPRESSION OF GH, GHR1 AND IGF-I GENES IN Oreochromis niloticus L FINGERLINGS

HASSAAN M.S.M.1,2, MOUSTAFA M.M.A.2,3, EL-GARHY H.A.S.2,3 AND REFAAT M.H.2,3

1Fish Nutrition Research Laboratory, National Institute of Oceanography and Fisheries (NIOF), Cairo, Egypt.
2Genetics Department, Fac. of Agriculture, Benha University, Qalubia, Egypt.
3Agricultural Biology Lab., Fac. of Agriculture, Benha University, Qalubia, Egypt.

*Corresponding Author: Email: Mohamed_shaban200065@yahoo.com

Received: March 15, 2014; Revised: April 09, 2015; Accepted: April 14, 2015

Abstract- A combination of probiotics and prebiotics as synbiotics allows assessing their synergistic effects. This study evaluated the effect of synbiotic (Lactobacillus acidophilus and fructooligosaccharides+mannan oligosaccharides, FMOS) on growth, hematological parameters, plasma hormonal, and genes expression in Oreochromis niloticus. A total of 600 O. niloticus of an average initial weight ranged from (4.96 to 5.96 g) was divided into four experimental groups for 84 days. Four basal diets Diet 1 (control), Diet 2, Diet 3 and Diet 4 were formulated to contain four levels of L. acidophilus (0.00, 0.42×10^6, 0.84×10^6 and 1.35×10^6 CFU g^-1) respectively, each level supplemented with 1% FMOS except of the control diet. O. niloticus fed diet supplemented with synbiotic showed significant (P<0.05) increases in growth and feed utilization. The highest final body weight, best feed conversion ratio, protein efficiency ratio and best chemical composition were obtained by the fish fed synbiotic Diet 3. Supplementation with synbiotic significantly increased in hematological parameters, growth hormone (GH) and insulin-like growth factor-I (IGF-I). The highest expression of GH and GHR1 were detected in liver of fish fed Diet 3. However, IGF-I was down regulated in liver of fish fed Diet 2 and Diet 4 whereas, IGF-I mRNA level in liver of fish fed Diet 3 up regulated and its expression was parallel with GH and GHR1 expression in liver of fish fed Diet 3. The expression of GH and GHR1 genes in spleen of fish fed Diet 3, Diet 2 and Diet 4. On contrary, the expression level of IGF-I in spleen of fish received either Diet 3 or Diet 4 was slightly up regulated, but IGF-I mRNA level was down regulated in fish fed Diet 2 than other treatments. The expression level of GH, IGF-I and GHR1 genes were down regulated in intestine of fish fed synbiotic than other control diet.

Keywords- Synbiotic, growth, gene expression, Oreochromis niloticus

Introduction

Tilapia is a worldwide fish of great commercial importance and it is recognized as one of the most important aquaculture species of the 21st century. The world’s total tilapia production in 2010 was 3.49 million tons [1]. Unfortunately, intensive aqua-farming is accompanied by several problems where the disease infection is a limiting factor for the production through the negative impact on growth. One of the main challenges to achieve productive, feasible and sustainable aquaculture is to develop alternative preventive practices that may help to maintain high animal welfare standards as well as healthy environment, resulting in a better production and higher profits. A novel approach to achieve the above mentioned goals is an application of probiotics and prebiotics in the fish farming industry[2-4]. In a practical sense, probiotics are defined as live microorganisms that are used as dietary supplements in aquaculture in the colon’ through the combination of probiotics and prebiotics in so-called synbiotics. Also they reported that the use of the synbiotics concept may give the benefit of both pre- and probiotics on fish growth. The synergistic effect may improve the survival of the probiotic organism, where the simultaneous presence of probiotic and prebiotic reward the host in a proper manner [8]. Few data are available regarding the application of synbiotics in aquaculture [9-11]. The research on the effects of synbiotic on levels of growth hormone (GH), insulin like growth factor I (IGF-I) and their gene expression in fish is very limited. In fish, growth is under the control of GH secretion from the pituitary, regulating somatic growth, organ and tissue growth and metabolic processes [12]. Most of these biological functions are mediated by plasma IGF-I, released from the liver in response to circulating GH [13]. Indeed, the hypothesis that the GH/IGF-I axis could be used as a marker of growth performance and nutritional status in aquaculture has already been suggested [14]. Thus, there is obviously a need to excess our knowledge of the effective preparation and safety valuation of synbiotics. Hence, this trial aimed to assess the effect of synbiotic with
different levels on growth performance, feed utilization, hematological parameters, plasma hormonal level of GH and IGF-I and GH, GHR1 and IGF-I genes expression in Nile tilapia (Oreochromis niloticus) fingerlings.

Material and Methods

Experimental Design and Culture Technique

Nile tilapia (Oreochromis niloticus) with an average initial body weight of (5.91±0.04 g) was obtained from Abbassa, Abo-Hammad, Sharkia Governorate, Egypt. The fish were acclimated for two weeks at fish research station, El-Kanater El-Khayria, National Institute of Oceanography and Fisheries (NIOF), Cairo, Egypt. During this period, fish were fed a commercial tilapia diet twice a day. The feeding experiment was conducted in 12 concrete ponds (0.5 m3 and 1 m depth). The ponds were supplied with fresh water from the Darawa irrigation branch, Kalubiya, Governorate using a pump machine and putting a fine net in inlet of each pond. Each pond was stocked with 50 fish. Three replicate units were randomly assigned to each treatment, prior to the start of the experiment and each pond was considered as an experimental unit. During the experiment, all fish were hand-fed their respective diets at a level of 3% of body weight 6 days a week. The daily ration was divided into three equal amounts and offered three times a day (09:00, 12:00 and 15:00 hours). A random sample of fish from each treatment was weighed biweekly and the amount of daily diet was adjusted accordingly. Freshwater in each pond was renewed 30% by the outlet at the bottom of the pond daily, before feeding. They were provided with continuous aeration to maintain the dissolved oxygen level near saturation, and fish were held under natural light.

Water temperature and dissolved oxygen were measured every other day using a YSI model 58 oxygen meter (YSI Company, Yellow Springs Instrument, Yellow Springs, Ohio, USA). Total ammonia and nitrite were measured twice weekly using a DREL, spectrophotometer (Deerfield, IL, USA). Total ammonia, nitrite, nitrate, carbon dioxide, pH, dissolved oxygen, temperature, and electrical conductivity were measured daily at 10:00 hours. A random sample of fish from each treatment was weighed biweekly and the amount of daily diet was adjusted accordingly. Freshwater in each pond was renewed 30% by the outlet at the bottom of the pond daily, before feeding. They were provided with continuous aeration to maintain the dissolved oxygen level near saturation, and fish were held under natural light.

Preparation Inoculum of L. acidophilus

L. acidophilus culture was prepared by adding 10 g of dried form (Microbiological Resources Center (MIRCEN), Faculty of Agriculture, Ain Shams Univ., Egypt) to 100 ml of prepared medium containing (gℓ-1): (peptone 5.0, beef extract, 3.0) broth and adjusted pH at 7.0 incubation was done at 37°C. After 24 h, 1 ml was inoculated into 100 ml fresh prepared medium broth that was incubated for a further 48 h at 37°C. After incubation, the cells were harvested by centrifugation (2000 g for 15 min), washed twice with phosphate buffered saline (PBS; pH 7.3; Oxoid) and re-suspended in PBS for addition to the basal diet.

Experimental Diets

The basal diet was formulated to contain approximately 30% crude protein and gross energy (19.41kJ g-1) which have been shown to be sufficient to support the optimal growth of O. niloticus. The basal diet was divided into four groups (Diet 1 (control), Diet 2, Diet 3 and Diet 4). Washed cells of Lactobacillus acidophilus were added dropwise into the basal diet mixture prior to pellet after to produce the probiotic diet with three levels 0.42×107, 0.84×107 and 1.35×107 respectively CFU g-1 [16]. The same volume of PBS (Lactobacillus acidophilus) was added to the basal mixture for the control. Each diet was supplemented with 1% (fructo-oligosaccharides and mannan oligosaccharides (FMOS) mixture which prepared with ratio 1:1, except diet1 (control). Fructo-oligosaccharides was purchased from (Encore Technologies, Plymouth, MN, USA.) and mannan oligosaccharides (Bio-Mos) purchased from (Alltech Inc., Nicholasville, KY, USA) [Table-1]. The ingredients were ground into fine powder through 200 µm mesh. All dry ingredients were thoroughly mixed with soybean oil, and then passing the mixed feed through a laboratory pellet mill (2-mm die) in National Institute of Oceanography and Fisheries, Cairo Governorate, Egypt (a California Pellet Mill, San Francisco, CA, USA), and stored at -20°C until use.

Table 1- Composition and proximate analysis of the experimental diets (% dry matter)

<table>
<thead>
<tr>
<th>Ingredients %</th>
<th>Diet NO.</th>
<th>L. acidophilus CFU g⁻¹</th>
<th>Fructooligosaccharide and mannanoligosaccharids (FMOS) %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diet 1</td>
<td>Diet 2 (0.42×10⁷ CFU g⁻¹)</td>
<td>Diet 3 (0.84×10⁷ CFU g⁻¹)</td>
</tr>
<tr>
<td>Fish meal</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>46</td>
<td>46</td>
<td>46</td>
</tr>
<tr>
<td>Yellow corn</td>
<td>29.5</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>10</td>
<td>10</td>
<td>9.4</td>
</tr>
<tr>
<td>soybean oil</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Vit. &amp; mineral¹</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>L. acidophulus²</td>
<td>0</td>
<td>0.1</td>
<td>0.15</td>
</tr>
<tr>
<td>FMOS³</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

¹Vitamin and mineral mix (mg or g / Kg diet): MnSO4, 40 mg; MgO, 10 mg; K2SO4, 40 mg; ZnCO3, 60 mg; KI, 0.4 mg; CuSO4, 12 mg; Fe2(Cr)2, 250 mg; Na2SeO3, 0.24 mg; Co, 0.2 mg; retinol, 40000 IU; cholecalciferol, 4000 IU; b-tocopherolacetate, 400 mg; menadione, 12 mg; thiamine, 30 mg; riboflavin, 40 mg; pyridoxine, 30 mg; cyancobalamin, 80 mcg; nicotinic acid, 300 mg; folic acid, 10 mg; biotin, 3 mg; pantothenic acid, 100 mg; inositol, 500 mg; ascorbic acid, 500 mg.

²Lactobacillus acidophilus was prepared to obtain (1.0×10⁷ CFU g⁻¹ approximately, Microbiological Resources Centre (MIRCEN), Faculty of Agriculture, Ain Shams Univ., Cairo, Egypt.).

³FMOS: 0.1% (fructo-oligosaccharides and mannan oligosaccharides) mixture which prepared with ratio 1:1. Fructooligosaccharides (Iclinil) purchased from (Encore Technologies, Plymouth, MN, USA.) and mannanoligosaccharides (Bio-Mos) purchased from (Alltech Inc., Nicholasville, KY, USA).

Proximate analysis

<table>
<thead>
<tr>
<th></th>
<th>Diet 1</th>
<th>Diet 2</th>
<th>Diet 3</th>
<th>Diet 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein %</td>
<td>30.05</td>
<td>29.92</td>
<td>29.91</td>
<td>29.9</td>
</tr>
<tr>
<td>Lipids %</td>
<td>5.69</td>
<td>5.65</td>
<td>5.65</td>
<td>5.64</td>
</tr>
<tr>
<td>Ash %</td>
<td>5.43</td>
<td>5.49</td>
<td>5.54</td>
<td>5.57</td>
</tr>
<tr>
<td>Total carbohydrate%</td>
<td>58.83</td>
<td>58.94</td>
<td>58.9</td>
<td>58.89</td>
</tr>
<tr>
<td>Gross energy (KJ g⁻¹)</td>
<td>19.45</td>
<td>19.42</td>
<td>19.41</td>
<td>19.4</td>
</tr>
</tbody>
</table>

Growth Parameters

Growth performance and feed conversion were measured in terms of final body weight (g), weight gain (WG), specific growth rate (SGR, % day⁻¹), feed conversion ratio (FCR), Protein efficiency ratio (PER) and feed intake. Growth response parameters were calculated on dry matter as follows:

The Influence of Synbiotic on Growth and Expression of GH, GHR1 and IGF-I Genes in Oreochromis niloticus L Fingerlings

Journal of Fisheries and Aquaculture
ISSN: 0976-9927 & E-ISSN: 0976-9935, Volume 6, Issue 1, 2015

177
Weight gain (WG) = final body weight (g) – initial body weight (g); Specific growth rate (SGR) =100× ([Ln (W2)-Ln (W1)]/T) Where: Ln = the natural log; W1 = initial body weight; W2 = final body weight and T = period of study (12 weeks); Feed conversion ratio (FCR) =Feed intake (FI) (g)/WG (g); Protein efficiency ratio (PER) = WG (g)/Protein intake (g).

Hemato logical Parameters and Hormonal Levels
At the end of the experimental feeding, ten fish were randomly collected from each treatment. Whole blood in each treatment was collected in Eppendorf tubes with anticoagulant (heparin 15 unit ml⁻¹) from the caudal vein of each fish. The blood sample was divided into two portions. The first one was used to determine hematocrit (Htc), haemoglobin (Hb), erythrocyte counts (RBCs) and total count of white blood cells (WBCs) according to standard methods as described elsewhere [17]. The second portion was centrifuged at 1000 x g for 5 min to separate the plasma. Plasma GH was measured by a radioimmunoassay (RIA) kit for Tianjin Nine Tripods Medical and Bioengineering Co., Ltd. (Tianjin, China), following the manufacturer’s protocol. Plasma IGF-I levels were determined in undiluted samples by RIA after SepPak C18 chromatography (Waters Corp., Milford, MA, USA), as described earlier for mammals [18].

Total RNA Extraction and Complementary Deoxyribonucleic Acid (cDNA) Synthesis
Liver, spleen and intestine samples were dissected from fish fed different diets and frozen at -80°C immediately until use. Tissue from each sample was ground by Tissue Lyser LT apparatus (Qiagen GmbH, QIAGEN Strasse 1, Hilden, Nordrhein-Westfalen-40724, and Germany) then total RNA was extracted from the suspension of cells using RNEasy® Mini kit (Qiagen) following the manufacturer’s protocol. DNase treatment was carried out to ensure that RNA samples were genomic DNA free. Then re-suspended in RNase-free water and quantified using Thermo Scientific Nano Drop 1000 Spectrophotometer (Thermo Scientific, USA). Single stranded cDNA was synthesized from 1000 ng of total RNA according to manufacturer’s protocol of High Capacity cDNA Reverse Transcription Kits (Applied Bio systems, Catalog number 4368813). Cycling conditions were: 25°C for 10 min, 37°C for 120 min and 85°C for 5 min. Then total RNA and cDNA samples were stored at -80°C until use.

Primers Design
Primers used in this study [Table-2] were created for GH, IGF-I and GHRI sourced using the software GenScript Online PCR Primers Design Tool based on Oreochromis niloticus and O. mossambicus mRNA sequences deposited in GenBank. The specificity of the primers was checked by alignments with the original GenBank sequences using the standard nucleotide-nucleotide BLAST (blast; provided online by NCBI). In this study 18s rRNA was selected as the reference gene for qPCR data normalization in the present study.

Quantitative Real Time PCR (qRT-PCR)
Triplicate PCR reactions were carried out for each analyzed sample. Each PCR reaction consisted of, 2.5μl of 1μg/μl cDNA, 12.5 μl SYBR Green PCR Master Mix (QuantiTect SYBR Green PCR Kit, Qiagen), 0.3 μM of each forward and reverse primer and double distilled water to a final volume of 25 μl. Reactions were then analyzed on an Applied Biosystem 7500 Real time PCR Detection system (Applied Bio systems) under the following conditions: 95°C for 10 min and 45 cycles of 95°C for 20 s followed by 60°C for 20 s and 72°C for 20 s. The fluorescence monitoring occurred at the end of each cycle. 18s RNA gene was used as reference gene for qPCR data normalization according to Shved [19].

Statistical Analysis of Treatment Effects
All experimentally induced changes in GH, IGF-I and GHRT expression are presented as n-fold changes (graphically depicted in %) relative to the corresponding controls set as 1 (100%). The comparative threshold cycle (ΔΔCₚ) method of Livak and Schmittgen [20] was used to calculate relative gene expression ratios as previously described [21]. Prior to analysis, qPCR assays were validated by plotting CT values against the logarithms of the dilution factors. Relative gene expression ratios (R) between treated and control groups were calculated using the formula: R = 2ΔΔC with ΔC = C_target - C_reference gene (target gene) -C_reference gene (treated group) - ΔC_reference gene (untreated control). All data are presented as means ± standard error (SE) and were analyzed using one way ANOVA, followed by Duncan’s [22] multiple range tests was used to compare differences among individual means, with statistical software SAS ANOVA procedure (statistical analysis system, 1993). A probability of 0.05 was utilized to account for the statistical difference between the means.

Results
Growth, Nutrient Utilization and Chemical Composition Indices
No mortality occurred during the entire experimental period. The indicators of growth performance and feed utilization were higher in O. niloticus fed synbiotic compared with control diet and the statistical analysis demonstrated significant differences (P<0.05) in growth performance and feed utilization. The greatest means of final body weight, WG and SGR were observed in fish fed Diet 3 [Table-3]. Significant enhancement (P<0.05) in feed intake (FI), protein efficiency ratio (PER) and feed conversion ratio (FCR) were recorded by fish fed the dietary synbiotic, in particularly with fish fed Diet 3 recorded the best indices [Table 3].

Concerning the influence of different dietary synbiotic levels on chemical proximate analysis of whole body fish [Table-4], dry matter, lipid, crude protein and ash contents of O. niloticus were significantly (P<0.05) influenced by the different treatments. Fish fed either Diet 3 or Diet 4 showed the highest lipid and crude protein, while fish fed Diet 3 was recorded higher ash content than other diet.
The Influence of Synbiotic on Growth and Expression of GH, GHR1 and IGF-I Genes in Oreochromis niloticus L Fingerlings

Table 3- Growth performance and nutrient utilization of O. niloticus after 84 days of feeding synbiotic-supplemented diets

<table>
<thead>
<tr>
<th>Experimental diet</th>
<th>Diet1</th>
<th>Diet2</th>
<th>Diet3</th>
<th>Diet4</th>
<th>±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g/ fish)</td>
<td>5.91± 0.06</td>
<td>5.83± 0.05</td>
<td>5.9± 0.05</td>
<td>4.96± 0.05</td>
<td>0.04</td>
</tr>
<tr>
<td>Final body weight (g/ fish)</td>
<td>34.70± 0.07</td>
<td>41.37± 0.06</td>
<td>44.10± 0.05</td>
<td>42.57± 0.05</td>
<td>0.145</td>
</tr>
<tr>
<td>Weight gain (g/ fish)</td>
<td>28.79± 1.29</td>
<td>35.53± 1.28</td>
<td>38.11± 1.27</td>
<td>36.61± 1.26</td>
<td>0.157</td>
</tr>
<tr>
<td>Specific growth rate (%/day)</td>
<td>1.97± 0.01</td>
<td>2.18± 0.01</td>
<td>2.22± 0.01</td>
<td>2.19± 0.01</td>
<td>0.008</td>
</tr>
<tr>
<td>Feed intake (g/ fish/period)</td>
<td>52.97± 0.02</td>
<td>56.32± 0.02</td>
<td>56.57± 0.02</td>
<td>56.53± 0.02</td>
<td>0.232</td>
</tr>
<tr>
<td>Feed conversion ratio</td>
<td>1.84± 0.01</td>
<td>1.95± 0.01</td>
<td>1.46± 0.01</td>
<td>1.54± 0.01</td>
<td>0.008</td>
</tr>
<tr>
<td>Protein efficiency ratio</td>
<td>1.84± 0.01</td>
<td>2.13± 0.01</td>
<td>2.32± 0.01</td>
<td>2.20± 0.01</td>
<td>0.013</td>
</tr>
</tbody>
</table>

-Values (± SE, N= 3). Means in within same row sharing the same superscript are not significantly different (P>0.05).

Hematological Parameters and Hormonal Levels

The effect of synbiotic on hematological and hormonal parameters is displayed in [Table 5]. Htc, Hb, RBC and WBCs in fish fed with different levels of synbiotic were significantly (P< 0.05) higher than the control. The highest values in Htc, Hb, RBC and WBCs were shown in Fish fed Diet 3.

Hormonal level for O. niloticus feeding different level of synbiotic showed significant rise (P<0.05) in growth hormone (GH) and insulin like-growth hormone factor-1 (IGF-I) in fish fed Diet 3 and Diet 4 and the highest values of GH and IGF-I were detected in fish fed Diet 3.

Table 4- Chemical composition of the whole carcass of O. niloticus after 84 days of feeding synbiotic-supplemented diets

<table>
<thead>
<tr>
<th>Experimental diet</th>
<th>Diet1</th>
<th>Diet2</th>
<th>Diet3</th>
<th>Diet4</th>
<th>±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>24.66± 0.01</td>
<td>24.29± 0.01</td>
<td>26.32± 0.01</td>
<td>24.50± 0.01</td>
<td>0.081</td>
</tr>
<tr>
<td>Crude protein %</td>
<td>55.29± 0.02</td>
<td>55.78± 0.02</td>
<td>56.35± 0.02</td>
<td>56.22± 0.02</td>
<td>0.166</td>
</tr>
<tr>
<td>Lipid %</td>
<td>14.48± 0.01</td>
<td>15.29± 0.01</td>
<td>15.65± 0.01</td>
<td>15.45± 0.01</td>
<td>0.123</td>
</tr>
<tr>
<td>Ash %</td>
<td>14.20± 0.01</td>
<td>14.67± 0.01</td>
<td>14.85± 0.01</td>
<td>14.43± 0.01</td>
<td>0.102</td>
</tr>
</tbody>
</table>

-Values (± SE, N= 3). Means in within same row sharing the same superscript are not significantly different (P>0.05).

Expression of IGF-I, GH and GHR1 Genes

Melting curves is an approach for validation of real-time PCR analysis and distinguishing between DNA fragments. During this study, the used primers gave a specific PCR product and there is no non-specific amplification as revealed from melting curve analysis. Also melting curves show that no contaminating products are present in this reaction, contaminating DNA or primer dimers would show up as an additional peak separate from the desired amplicon peak. The effect of synbiotic with different levels on differential expression and regulation of IGF-I, GH and GHR1 genes in different organs, liver, spleen and intestine in Nile tilapia, by SYBR-Green real-time PCR assay showed in [Fig 1], [Fig 2] & [Fig 3].
showed that rainbow trout fed diets supplemented with mannan oligosaccharides (MOS), (Enterococcus and MOS) and (Enterococcus, MOS and polyhydroxybutyrate acid, PHB) recorded significantly higher WG and SGR than those of the rest experimental groups. Ye [30] reported that, Japanese flounder fed diet supplemented with (fructo-oligosaccharides (FOS), MOS and Bacillus clausii) increased FBW and WG than other diets. Also, Al [31] showed that at each dietary FOS level, dietary supplementation of 1.35×10^7 CFUg^-1 B. subtilis significantly increased the SGR compared with the groups without B. subtilis supplementation in juvenile large yellow croaker, Larimichthys crocea. Similarly, Mehrabi [32] reported that, rainbow trout (Onchorhynchus mykiss) fingerlings fed diets supplemented with different levels of synbiotic (Enterococcus faecium/ FOS) showed increase in growth performance in comparison with the control group. Montajami [33] reported that Texas cichlid larvae (Herichthys cyanoguttatus) fed the synbiotic had significantly increased final body weight in comparison to control treatment (P<0.05). The minimal FCR of the fish in this study was detected with a dietary synbiotic of (0.84×10^9 CFUg^-1 and 1%). This may suggest that O. niloticus is able to utilize food efficiently while receiving relatively medium level of synbiotic, which, in turn, would be more beneficial, also, synbiotic may serve in this case as a co-feeding of inert feed and may help to maximize the diets efficiency through stimulating digestive tract. Co-feeding not only stimulates the ingestion of feed particles, but also promotes digestion and assimilation of diets by fish [34]. Feed conversion ratio is considered to be one of the economic benefits of aquaculture because, in addition to reduction in feeding costs due to decreased feeding, it prevents deteriorating of the cultivation media and, as a result, degradation of water quality eventually leading to increased profits [35].

Discussion

Manipulation of gastrointestinal tract microbiota through probiotic and/or prebiotics dietary supplementations is a novel approach from nutritional point of view and an alternative for antibiotics and immunity promotion. Recently, probiotics and prebiotics have become an integral part of the aquaculture practices for improving the growth performance [23,24].

Synbiotics, the combined application of probiotics and prebiotics, is based on the principle of providing probiotics with a competitive advantage over competing endogenous populations; thus, effectively improving the survival and implantation of the live microbial dietary supplement in the gastrointestinal tract of the host [7]. The use of synbiotics it may be possible to produce greater benefits than the application of individual probiotics [25].

In the present study, growth performance and feed utilization of O. niloticus were enhanced significantly by synbiotic (0.84×10^9 CFUg^-1 and 1%) and (1.35×10^7 CFUg^-1 and 1%) supplementation. This result may be attributed with Gibson and Robefroid [7] they concluded that a combination between probiotic and prebiotic could improve the survival of the probiotic organism because fermentation can be implemented more effectively as its required specific substrate is readily available. Simultaneous presence of probiotic and prebiotic, therefore, benefits the host in a proper manner [8]. Furthermore, the obtained results may be due to the effect of synbiotic that inhibit the colonization of potential pathogens in the digestive tract by antibiosis or by competition for nutrients and space, and alteration of the microbial metabolism. It also improves the nutrition by detoxifying the potentially harmful compounds in feeds, by producing vitamins such as biotin and vitamin B12 [26], and by stimulating host immunity [27]. Another possible explanation for increased growth performance with added probiotic is the improvement in digestibility, which may in turn explain the better growth and feed efficiency observed with the supplemented diets. Otherwise, probiotics influence digestive processes by enhancing the population of beneficial microorganisms, microbial enzyme activity, improving the intestinal microbiotal balance, consequently improving the digestibility and absorption of food and feed utilization [28]. Recently, Firouzbakhsh [29] reported that O. mykiss fingerlings fed diets containing Enterococcus faecium as probiotic, and Fructooligosaccharide (FOS) as prebiotic significantly showed higher WG and SGR. Rodriguez-Estrada [10]
plies the concentration of hemoglobin ultimately resulting in a high capacity for oxygen carrying in the probiotic-fed fish. Rodriguez-Estrada [10] found that Htc value was higher in the (Enterococcus and MOS) and (Enterococcus, MOS and PHB) groups than the other groups.

The significant (P<0.05) increasing in the WBCs of fish which fed symbiotic (0.84×10^7/1%) in the present study compared with control. Similar findings were reported by Firouzbakhsh [29] who showed that the highest (P < 0.05) WBC was recorded by the fish fed a diet of 1 g/ kg symbiotic for two months. Reinforcement of non-specific immune system as a result of probiotic consumption can be a possible explanation of the elevated number of WBC [23]. Also, Irianto and Austin [2] found in rainbow trout (Oncorhynchus mykiss) and Firouzbakhsh [37] found in Oscar (Astronotus ocellatus) that WBC was increased especially lymphocytes following the use of probiotic.

In fish, growth is under the control of growth hormone (GH) secretion from the pituitary, regulating somatic growth, organ and tissue growth and metabolic processes that influence somatic growth [12]. Most of these biological functions are mediated by plasma insulin-like growth factor I (IGF-I), released from the liver in response to circulating GH [13]. Indeed, the hypothesis that the GH/IGF-I axis could be used as a marker of growth performance and nutritional status in aquaculture has already been suggested [14]. Also GH may be acting in an endocrine and paracrine fashion within and between neighboring cells to stimulate IGF-I which may in turn act in an autocrine or paracrine manner to stimulate growth [38,39]. In the present study, the dietary of synbiotic was enhanced and regulated physiological status of the experimented fish based on the significant change records in the Plasma GH and IGF-I levels compared with control fish. Growth hormone (GH) initiates many of its growth-promoting actions by binding to GH receptors (GHRs) and stimulating the synthesis and secretion of insulin-like growth factor-I (IGF-I) from the liver and other sites [40]. At the same time, at the molecular level, the expression of genes involved in muscular growth was also positively affected by bacterial integrators confirming a beneficial role of synbiotic on the whole metabolism. Furthermore, the higher expression level of GH, IGF-I and GHR1 genes in liver tissues obtained from fish fed Diet 3 than other organ. This indicated that spleen and intestine tissues are not specific organs to express GH and GHR1, but, the specific organ to express IGF-I gene is liver followed by spleen tissues. Carnavalli [41] showed that sea bass juveniles (Dicentrarchus labrax) fed on probiotics showed significantly higher IGF-I expression with respect to control group. IGF-I is extremely important for the regulation of the establishment and the maintenance of differentiated cell functions via endocrine and paracrine, autocrine signaling [42], as well as the promotion of cellular proliferation and differentiation in many systems [43]. An explanation for this apparent gene expression level in the liver under our treatments remains elusive. The extent of feeding might affect GHR expression [44]. Such studies are vital for understanding the differential regulation of expression of these growth factors under the studied treatments and would help us to delineate the biological significance of these growth factors in O. niloticus.

Conclusions
Considering the low cost of production of L. acidophilus for aquaculture which offer convenience and cost benefits to farm operators and on the basis of the data here obtained, we suggest that (symbiotic) composed by L. acidophilus, fructo-oligosaccharides and mannan oligosaccharides as a valuable feed additive in O. niloticus L fingerlings. Specially, (0.84×10^7 CFU g⁻¹/1%) and (1.35×10^7 CFU g⁻¹/1%) enhance significantly growth parameters, feed utilization, Plasma GH and IGF-I levels and the expression level of GH, GHR and IGF-I genes in liver and spleen. The results of this study provided new insight for emerging synbiotic biotechnology, for further increase of productivity and competitiveness of the aquaculture industry.

Abbreviations
GH: Growth hormone
GHR1: Growth hormone receptor-1
IGF-I: Insulin like growth factor-1
FMOS: fructooligosaccharides+mannan oligosaccharides
CFU: colony-forming unit
mRNA: Messenger Ribonucleic Aid
FAO: Food and Agriculture Organization
WG: weight gain
SGR: specific growth rate
FCR: feed conversion ratio
PER: Protein efficiency ratio
Fi: Feed Intak
Htc: hematocrit
Hb: haemoglobin
RBCs: erythrocyte counts
WBCs: total count of white blood cells
cDNA: DNA complementary to RNA
Ct: threshold cycle
rRNA: ribosomal RNA
DNase: deoxyribonuclease
FBW: final body weight
qRT-PCR: Quantitative real time PCR

Conflicts of Interest: None declared.

References

Journal of Fisheries and Aquaculture ISSN: 0976-9927 & E-ISSN: 0976-9935, Volume 6, Issue 1, 2015
Bioinfo Publications


