Effects of Dietary Inulin as Prebiotic on Growth Performance, Immuno-haematological Indices and Ectoparasitic Infection of Fingerlings Nile Tilapia, Oreochromis Niloticus

Original Article

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ABSTRACT

Background: Prebiotics contain non-digestible ingredients, which beneficially affect to the host by selectively stimulating the growth of one or a limited number of bacteria at the colon. Inulin and oligofructose are the most commonly used as prebiotic food ingredients in aquaculture. Dietary supplemented of inulin has shown an enhancement on growth and health of fish.

Aim of the Study: The current study aimed to assess the effects of varying doses of inulin as a natural prebiotic on the growth, nutrient utilization, liver histology, immune responses and ectoparasitic infection of fingerlings Nile tilapia (Oreochromis niloticus).

Materials and Methods: Eighteen full glass aquaria measuring (75×40×35cm) were distributed into six treatments and each treatment was represented in three replicates (25 fish in each aquarium with an initial body weight of 9.23±0.25g). Six diets were formulated by using two levels of sorghum (15 and 30%) and the inulin, which produced and imported from China was used in each level with three doses 2.5,5.0 and10.0g/kg diet. Fish samples of (blood, histological examination of liver and spleen, ectoparasitic infection and proximate composition of fish) were detected.

Results: The results demonstrated that the highest growth performance and feed efficiency were obtained with dietary low inulin ratio (2.5 g/kg diet). White Blood Cell (WBC), Alternative Complement Pathway (ACP) and Superoxide Dismutase Activity (SOD) were affected significantly (P˂0.05) with increased dietary inulin doses. Results of histopathological examinations showed that the fish fed in low dose of inulin improve liver and spleen structures. Also, the ectoparasitic infection showed enhancement in resistance for ectoparasatic with all doses of inulin.

Conclusion: The present results showed that inulin supplementation at low dose (2.5g/kg diet) in the two levels of sorghum inclusion (15 & 30%) in dietry Nile Tilapia, enhancing growth performance, immuno-haematological indices, parasitic infection and each of liver and spleen structure of Nile tilapia fingerlings.

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Key Words: Ectoparasitic infection, growth performance, inulin prebiotic, lysozyme activity, nile tilapia.

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INTRODUCTION

Nile tilapia, Oreochromis niloticus is one of the most economic farmed species, distributed in different site of the world, can persist in different environmental conduction and constitutes more than 80% of fish production in Egypt[1,2]. The efficiently to digest and metabolic carbohydrate in different fish species are different, but in general, herbivorous fish can use high level of carbohydrate in their diets than omnivorous and carnivorous fish[3].

Sorghum is one of the most adapted summer grain crops to drought and heat. In Egypt, Sorghum is grown in Upper Egypt from Giza to Aswan, but most of the area (89 thousand hectare) is concentrated in Assiut and Sohag governorates and about 37 thousand hectare in Fayoum governorate. It ranks the fifth of the world cereal crops after wheat, rice, maize and barley[4]. World global sorghum production in 2018 was 60 million metric tons and their produced in Egypt is 750.000 metric tons[5,6]. Sorghum is similar in their composition to maize, but can contain some ant-nutritional factors, which can inhibit growth in fish. However, by using some technological process as drying, soaking and grinding before fed can enhance its nutritional value. Moreover, it's feasible to replace imported and expensive crops as maize[7].

Disease is considered the end consequence of a complex interaction in the host. However, the environment, pathogen itself, and capacity of impedance to various diseases are often as a crucial factor affecting cultured species[8,9]. In addition, anti-biotic drugs can also destroyed beneficial bacteria[10] and increase the indulgence of pathogenic bacteria[11]. Many researchers concluded that probiotics were useful in the growth performance and immune function of aquatic animals[12,13].

From a long time, antibiotic agents were commonly applied for restrain bacterial diseases in commercial aquaculture projects[14]. In 2006, the European Union
prohibit the utilization of antibiotics as feed additives\(^{(16)}\). So, the search for ersatz, uncommon environmentally treatments and control strategies has become imperious\(^{(17)}\). One of the alternatives to antibiotics is the use of prebiotics, where its management has been displayed as convenient strategies for adjustment the immune response and enhance disease resistance in fish\(^{(18)}\). Arranging of prebiotics has been cleared to increase the positive effect on beneficial gut bacteria as lactic acid bacteria (LAB), which are benefits for the host\(^{(19)}\).

Prebiotics contain non-digestible ingredients, which beneficially affect to the host by selectively stimulating the growth of one or a limited number of bacteria at the colon\(^{(20)}\).

Inulin and oligofructose are the most commonly used as prebiotic food ingredients in Europe. They are fructans that can be present in dahlia, chicory, Jerusalem artichokes, garlic and to a lesser value in cereals\(^{(21)}\). Dietary supplemented of inulin has shown an enhancement on growth and health of fish\(^{(22,23)}\).

The expanding in aquaculture technology led to an increasing need for improved diagnostic methods. Hematology and clinical chemistry analysis, although not used regularly in fish medicine evaluation, but it's provide substantial diagnostic information once reference values are confirmed\(^{(24)}\).

Therefore, the current study aimed to assess the effects of varying doses of inulin as a natural prebiotic on the growth, nutrient utilization, liver histology, immune responses and ecotoparasitic infection of fingerlings Nile tilapia (Oreochromis niloticus).

**MATERIALS AND METHODS**

**Experimental Units**

Eighteen full glass aquaria measuring (75×40×35cm) were used in the growth trial. Aerated de-chlorinated water was used in fish rearing and the water changed continuously. Each aquarium was supplied with air pump to maintain good aeration. Thermostatic heaters were used during the experiment to maintain temperature at 25±2°C.

**Experimental Fish**

Fingerlings of Oreochromis niloticus were brought from Fish Research Centre, Suez Canal University, where it put in strong plastic bags filled with natural fresh water and oxygen, respectively. Fish were divided into eighteen groups representing six experimental treatments and reared for two weeks on experimental diets. Each treatment was represented in three replicates with a stocking density of 25 fish in each glass aquarium and an initial body weight of 9.23 ± 0.25g.

**Experimental Diet**

The experimental diets were formulated to cover the nutritional requirement of this specie by using available feed ingredients. Feed ingredients were grinded into fine powder through a 0.6mm mesh sieve. The mixed diet was made into soft dough by added the available local ingredients. The mixture was pelleted using California pelleting machine with a diameter of 2mm and air dried. Six diets were formulated by using two levels of sorghum (15 and 30%) and the inulin, which produced and imported from China was used in each level with three doses 2.5, 5.0 and 10.0g/kg diet. The diets were given to sex groups of fish as: group 1 contain inulin 2.5g and sorghum 15% (In 2.5g + S15%), group 2, inulin 5.0g and sorghum 15% (In5.0g + S15%), group 3, inulin 7.5g and sorghum 15% (In 7.5g + S15%), group 4, inulin 2.5g and sorghum 30% (In 2.5g + S30%), group 5, inulin 5.0g and sorghum 30% (In 5.0 + S30%) and group 6 inulin 7.5g and sorghum 30% (In 7.5g + 30%). The chemical analysis of diets are elucidated in (Table 1). Diets were fed to apparent visual satiation in two times daily (10:00 and 16:00 h) for a period of 90 days from (July to September, 2016). Fish were weighted every two weeks to adjust the amount of feed consumed during the experimental study.
EFFECT OF DIETARY INULIN ON NILE TILAPIA

Table 1: Feed formulation and chemical analysis of experimental diets

<table>
<thead>
<tr>
<th>Diets</th>
<th>Sorghum and Inulin levels %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S15</td>
</tr>
<tr>
<td>Fish meal</td>
<td>4</td>
</tr>
<tr>
<td>Poultry-by product meal</td>
<td>8</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>10</td>
</tr>
<tr>
<td>Sunflower seed meal</td>
<td>50</td>
</tr>
<tr>
<td>Sorghum meal</td>
<td>15</td>
</tr>
<tr>
<td>Fish oil</td>
<td>3</td>
</tr>
<tr>
<td>Cotton seed oil</td>
<td>3</td>
</tr>
<tr>
<td>Microcrystalline cellulose</td>
<td>5</td>
</tr>
<tr>
<td>Inulin</td>
<td>0.25</td>
</tr>
<tr>
<td>Vitamin min. mix</td>
<td>1.75</td>
</tr>
</tbody>
</table>

Chemical composition (%DM basis)

- Dry matter: 92.4, 92.1, 92.4, 92.1, 92.4, 92.1
- Crude protein: 30.37, 30.09, 30.37, 30.09, 30.37, 30.09
- Ether extract: 13.57, 12.82, 13.57, 12.82, 13.57, 12.82
- Nitrogen free extract: 35.22, 40.42, 35.22, 40.42, 35.22, 40.42
- Crude Fiber: 7.72, 6.47, 7.72, 6.47, 7.72, 6.47
- Ash: 13.12, 10.2, 13.12, 10.2, 13.12, 10.2
- Gross energy (MJ/kg diet): 18.84, 18.78, 18.84, 18.78, 18.84, 18.78
- Metabolizable (ME/kg diet): 15.74, 16.19, 15.74, 16.19, 15.74, 16.19

1. The experimental diets used in this trial after (Yones et al., unpublished results).
2. Vitamin-mineral premix supplied the following (g Kg-1 mixture); retinyl acetate 0.67; ascorbic acid 120; cholecalciferol 0.1; tocopheryl acetate 4.5; calcium pantothenate 14.1; p-aminobenzoic acid 0.03; menadione 30; biotin 0.1; choline chloride 350; folic acid 1.5; inositol 50; canthaxanthin 10; butylated hydroxytoluene 1.5; butylated hydroxyanisol 1.5; CaHPO4,2H2O 29.5; Ca(H2PO4)2·H2O 217; NaHCO3 94.5; Na2SeO3·H2O 0.011; Kci 100; NaCl 172.4; K 0.2; MgCl2 63.7; MgSO4 34.3; MnSO4 2; FeSO4·H2O 10; CuSO4·5H2O 0.4; ZnSO4 10.
3. Gross energy (MJ/kg-1) was calculated by using the following calorific values: 23.9, 39.8, and 17.6 kJ/g dietary protein, crude lipid and nitrogen free extract, respectively.
4. Metabolizable energy (MJ/kg-1) was calculated by using the following calorific values: 18.9, 35.7, and 14.7 kJ g-1 diet for protein, ether extract and nitrogen free extract, respectively.

Growth Parameters

Growth indices were measured as the following equations:

- Weight body weight gain (WG) = (FW − IW).
- FW = Final weight (g).
- IW = Initial weight.

Specific growth rate SGR (%/day) = (Ln FW − Ln IW)/T × 100.

- Ln = Natural logarithm
- T = period (days).
- Condition factor (K) = W/L3 × 100.

Where: W = fish weight (g); L = fish total length (cm).

- Feed conversion ratio (FCR) = Feed intake (g)/Weight gain (g).

- Protein efficiency ratio (PER %) = Weight gain (g)/Protein intake (g).

- Net protein utilization (NPU%) = (Final body protein - Initial body protein) × 100/Protein intake.

- Hepatosomatic index = Liver weight (g) × 100/fish body weight (g).

Chemical Analysis

Analyses of diets and carcass composition were made according to standard (AOAC) methods. The moisture was determined by drying samples in Japanese oven (Labostar-LG 122, Tabai Espec, Osaka, Japan) on 105 0C for 24 h; ash by burning in a muffle furnace (Isuzu Seisakusho, Tokyo, Japan) at 550 0C for 18 h; crude protein by the Kjeldahl method (N × 6.25) using an automatic Kjeldahl System (Buchi 430/323, Flawil, Switzerland), and crude lipid by using chloroform/methanol value of (2:1, v/v) extract procedure. Contents of tannins in sorghum meal were determined using a modified version method.
Red blood cell count, White blood cell count, haematocrit and haemoglobin content were detected through the method described by\cite{35}.

**Immunological Indices**

The lysozyme activity level was determined by the turbidimetric assay\cite{36}. Alternative complement pathway (ACP) was performed as described by\cite{37}. Superoxide dismutase activity (SOD) was determined as described by\cite{38}.

**Histological Examination**

After blood sampling, the fish were desiccated; liver and spleen were removed and prepared for further studies. Tissue specimens of each liver and spleen were fixed in (neutral buffered formalin 10%) for 24 hr. The fixed tissues were swill in fresh tap water, exsiccated through graded range of alcohols, cleared in two changes of xylene and fixed in paraffin wax\cite{39}. 5 µm thick sections were cut and soiled with Hematoxylin and Eosin (H & E) and the tissues were examined by light microscopy with magnification power (H&E,× 40).

**Ectoparasitic Infection with Examinations**

The ectoparasitic trial was done after finishing the growth trial, where 20 fish from each treatment were exposed to ectoparasitic test through 15 days. The ectoparasitic infection was induced through cohabitation method. In this method initial parasite burden of donor fish (D) was determined by examined fish before starting the cohabitation, and receptor fish (R) marked with tags to differentiate them. The test involved groups were fed on the same treatments used in growth trial. All groups were fed on the different levels of sorghum and inulin diets as presented in (Table 1). By ending 15 days of feeding on the experimental diets, aquarium water capacity have been decreased to 40 L and still not cleaned to increase stress on the experimental diets. The initial parasite burden of donor (D) was determined by examined fish before starting the experiment. As can be seen, the highest significance \( (P<0.01) \) values of (final weight, weight gain, specific growth rate and conduction factor) were obtained with the fish fed on diet (S15+2.5g inulin) compared with the other diets. However, less significance value from the mentioned parameters was found in the group fed on (S 30+7.5g inulin). Also, less significance values were recorded with the groups fed on (S30+2.5g, S30+5.0g and S30+7.5g ) diets, respectively.

In the same vein, the feed utilization represented in feed conversion ratio (FCR), protein efficiency ratio (PER) and net protein utilization (NPU) were significantly differences \( (P<0.05) \) between different doses of inulin and sorghum levels. Regarding the data in the same table the elevated values for feed utilization was reported in group of fish fed diet (S15+2.5g inulin) followed by (S15+5g, S15+7.5g, S30+2.5g,S30+5.0g and S30+7.5g) diets, respectively. No significance differences were found in hepatosomatic index (HSI) values either inulin or sorghum levels. The best highest significance values for growth performance and feed utilization were represented with the added of inulin as 2.5 g /kg diet with 15% sorghum level compared with the other treatments.

**Haematological Parameters**

No significance difference was obtained in RBCs between different diets. However, significance \( (p<0.05) \) differences were detected in WBCs, Ht and Hb for the different tested diets as presented in (Table 3). The data showed highly significant difference between diets \( (P<0.01) \) when compared after 90 days from the beginning of the experiment. As can be seen, the highest significance \( (P<0.01) \) values of (final weight, weight gain, specific growth rate and conduction factor) were obtained with the fish fed on diet (S15+2.5g inulin) compared with the other diets. However, less significance value from the mentioned parameters was found in the group fed on (S 30+7.5g inulin). Also, less significance values were recorded with the groups fed on (S30+2.5g, S30+5.0g and S30+7.5g ) diets, respectively.

**Statistical Analysis**

The current data of the present study were analyzed using two-ways Analysis of Variance (ANOVA) system for testing significance among groups\cite{38}. The comparisons between values were done by Duncan's Multiple Range test according to\cite{39}. All analysis were performed using SPSS version 20,(2016) SPSS Institute, Cary, NC, USA\cite{40}. Results are considered significantly at significant levels of 0.05, \( (P<0.05) \).

**RESULTS**

**Growth Performance**

The use of inulin as a prebiotic was reflected on the obtained parameters of growth performance in O. niloticus as presented in (Table 2). The data showed highly significant difference between diets \( (P<0.01) \) when compared after 90 days from the beginning of the experiment. As can be seen, the highest significance \( (P<0.01) \) values of (final weight, weight gain, specific growth rate and conduction factor) were obtained with the fish fed on diet (S15+2.5g inulin) compared with the other diets. However, less significance value from the mentioned parameters was found in the group fed on (S 30+7.5g inulin). Also, less significance values were recorded with the groups fed on (S30+2.5g, S30+5.0g and S30+7.5g ) diets, respectively.

In the same vein, the feed utilization represented in feed conversion ratio (FCR), protein efficiency ratio (PER) and net protein utilization (NPU) were significantly differences \( (P<0.05) \) between different doses of inulin and sorghum levels. Regarding the data in the same table the elevated values for feed utilization was reported in group of fish fed diet (S15+2.5g inulin) followed by (S15+5g, S15+7.5g, S30+2.5g,S30+5.0g and S30+7.5g) diets, respectively. No significance differences were found in hepatosomatic index (HSI) values either inulin or sorghum levels. The best highest significance values for growth performance and feed utilization were represented with the added of inulin as 2.5 g /kg diet with 15% sorghum level compared with the other treatments.

**Haematological Parameters**

No significance difference was obtained in RBCs between different diets. However, significance \( (p<0.05) \) differences were detected in WBCs, Ht and Hb for the different tested diets as presented in (Table 3).

**Immunological Indices**

The effects of different dietary doses of inulin addition on the immune responses of tilapia are shown in (Table 4). The lysozyme activity values were varies among the two levels of sorghum. The highest values were obtained with using 15% sorghum at the three doses of inulin and less significance values were revealed with30% sorghum diet. Significantly \( (p<0.05) \) highest Alternative complement pathway (ACP) and Superoxide dismutase activity (SOD) activity were observed in fish fed at high doses 7.5 g/kg.
of inulin in the two sorghum levels compared to the rest of tested diets. Histopathology in liver feeding 2.5g inulin showed that in group (a-15% sorghum) a mild focal hepatic vascular degeneration and (b-30% sorghum) showed mild congestion of blood vessels and vacuolation of hepatocytes observed (plate 7). On the other hand, fish fed with inulin 5g/kg diet showed in (a-15% sorghum) a moderate focal to diffuse degeneration of hepatocytes and (b-30% sorghum) showed severely congested blood vessels and diffuse vascular degeneration of hepatic cells (plate 8). On the same manner, fish fed with inulin 7.5g/kg diet (a-15% sorghum) showed severe degeneration of hepatocytes and congestion of blood vessels and (b-30% sorghum) showed severe degeneration of hepatocytes, focal hemorrhage and edema (plate 3).

**Histopathological Findings**

**Liver**

Histopathology in liver feeding 2.5g inulin showed that in group (a-15% sorghum) a mild focal hepatic vascular degeneration and (b-30% sorghum) showed mild congestion of blood vessels and vacuolation of hepatocytes observed (plate 1). On the other hand, fish fed with inulin 5g/kg diet showed in (a-15% sorghum) a moderate focal to diffuse degeneration of hepatocytes and (b-30% sorghum) showed severely congested blood vessels and diffuse vascular degeneration of hepatic cells (plate 2). On the same manner, fish fed with inulin 7.5g/kg diet (a-15% sorghum) showed multi focal to diffuse degeneration of hepatocytes and congestion of blood vessels and (b-30% sorghum) showed severe degeneration of hepatocytes, focal hemorrhage and edema (plate 3).

**Spleen**

In group fed 2.5 g inulin (a-15% sorghum) showed normal structure of both white and red pulp with active prominent macrophage pulp and (b-30% sorghum) showed fairly normal structure of both white and red pulp (plate 4). However, in fish fed with inulin 5g/kg diet (a-15% sorghum) showed normal splenic structure and (b-30% sorghum) showed mild depletion of lymphocytes with mild atrophied melanomacrophage centres (plate 5). Consequently, fish fed inulin 7.5g (a-15% sorghum) showed moderate congestion of blood vessels of red pulp and (b-30% sorghum) showed depletion and necrotic changes of white pulp and sever congestion of blood vessels and sinusoids (plate 6).

**Clinical Examinations**

The most characteristic of clinical findings observed on naturally infected (Oreochromis niloticus) fish were found as : abnormal swimming, flashing and rubbing their external bodies on the side of the aquaria. Also, suffered from asphyxiat, gathered at the water surface with gulping the atmospheric air. Some fish accumulated towards the air pumps and become nervous and irritable. The skin appeared with localized focal bloody spots with small wounds or abrasions. The mentioned signs were decreased in all treated fish with inulin compared with the control which non treated with inulin.

**Parasitic Examination**

As shown in (Table 5) the examination fish with Cichlidogyrus tilapia were transported to the treated fish groups with varying inulin doses. There were seven treated groups feeding on different levels and doses of sorghum and inulin. Each group contained 10 fish of treated act as receptor (R) and 10 examined fish with Cichlidogyrus tilapia parasite act as a donor (D). After two weeks of cohabitation between donor and receptor fish, examined and recorded infected fish showed that the lower values were recorded by using inulin in the three doses.

**Body Composition**

Body composition values were shown in (Table 6), where dry matter, crude protein and ash not shown significantly different (P>0.05) among experimental diets. However, the contents of lipid showed significance increased values (P<0.05) with the high doses of inulin as detected in fish fed (S30+5.0g and S30+7.5g inulin) compared with the other diets.
Table 2: Growth indices, feed efficiency and hepatosomatic index of Nile tilapia after feeding on different inulin and sorghum diets (Mean±SD n=3)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S15+2.5 g</td>
</tr>
<tr>
<td>Initial weight (g/fish)</td>
<td>9.31±0.25</td>
</tr>
<tr>
<td>Final weight (g/fish)</td>
<td>63.63±2.25</td>
</tr>
<tr>
<td>Total weight gain (g/fish)</td>
<td>54.30±0.32</td>
</tr>
<tr>
<td>Specific growth rate</td>
<td>2.13±0.04</td>
</tr>
<tr>
<td>Condition factor (g/cm3)</td>
<td>1.86±0.17</td>
</tr>
<tr>
<td>Survival rate (%)</td>
<td>99</td>
</tr>
<tr>
<td>Feed consumed(g)</td>
<td>83.0</td>
</tr>
<tr>
<td>Feed conversion ratio</td>
<td>1.53±0.35</td>
</tr>
<tr>
<td>Protein efficiency ratio</td>
<td>2.15±0.23</td>
</tr>
<tr>
<td>Net protein Utilization (%)</td>
<td>33.48±0.22</td>
</tr>
<tr>
<td>Hepatosomatic index (HSI %)</td>
<td>1.8±0.11</td>
</tr>
</tbody>
</table>

Means with different superscripts letters are significantly different (P<0.05)

Table 3: Haematological index at Nile tilapia after feeding on different inulin and sorghum diets (Mean±SD n=3)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S15+2.5 g</td>
</tr>
<tr>
<td>Red blood cell, RBC×10⁹/L</td>
<td>1.91±0.1</td>
</tr>
<tr>
<td>White blood cell, WBC×10¹²/L</td>
<td>96.2±1.2</td>
</tr>
<tr>
<td>Haematocrit, Hct/%</td>
<td>32.0±0.8</td>
</tr>
<tr>
<td>Haemoglobin, Hb g/L</td>
<td>7.2±0.2</td>
</tr>
</tbody>
</table>

Means with different superscripts letters are significantly different (P<0.05)

Table 4: Immunological indices at Nile tilapia after feeding on different inulin and sorghum diets (Mean±SD n=3)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S15+2.5 g</td>
</tr>
<tr>
<td>Lysozyme activity (mg/dl)</td>
<td>1.99±0.2</td>
</tr>
<tr>
<td>Alternative complement pathway (ACP) (Um/L)</td>
<td>65.0±1.4</td>
</tr>
<tr>
<td>Superoxide dismutase (SOD)</td>
<td>50.0±1.2</td>
</tr>
</tbody>
</table>

Means with different superscripts letters are significantly different (P<0.05)

Table 5: Effect of sorghum supplemented with inulin on Cichlidogyrus tilapia and morbidity of fish

<table>
<thead>
<tr>
<th>Treatments</th>
<th>No of treated fish (R) non infected</th>
<th>No of donor fish (D) infected</th>
<th>No of infested fish from R and D</th>
<th>Morbidity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>10</td>
<td>9</td>
<td>45</td>
</tr>
<tr>
<td>S15+2.5</td>
<td>10</td>
<td>10</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>S15+5.0</td>
<td>10</td>
<td>10</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>S15+7.5</td>
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<td>S30+2.5</td>
<td>10</td>
<td>10</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>S30+5.0</td>
<td>10</td>
<td>10</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>S30+7.5</td>
<td>10</td>
<td>10</td>
<td>3</td>
<td>15</td>
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</table>
Table 6: Proximate chemical composition of Nile tilapia after feeding on different inulin and sorghum diets (Mean±SD n=3)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatments</th>
</tr>
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<tbody>
<tr>
<td>Dry matter</td>
<td>S15+2.5 g</td>
</tr>
<tr>
<td></td>
<td>S15+5.0g</td>
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<tr>
<td></td>
<td>S15+7.5g</td>
</tr>
<tr>
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<td>S30+2.5g</td>
</tr>
<tr>
<td></td>
<td>S30+5.0g</td>
</tr>
<tr>
<td></td>
<td>S30+7.5g</td>
</tr>
<tr>
<td>Crude protein</td>
<td>26.92±0.42</td>
</tr>
<tr>
<td></td>
<td>26.85±0.38</td>
</tr>
<tr>
<td></td>
<td>27.53±0.39</td>
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<td>26.51±0.28</td>
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<td>28.35±0.34</td>
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<td>28.58±0.15</td>
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<td>Crude lipid</td>
<td>15.62±0.21</td>
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<td>15.42±0.54</td>
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<tr>
<td>Ash</td>
<td>6.15b±0.83</td>
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<td>5.04±0.19</td>
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<td>5.11±0.28</td>
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Means with different superscripts letters are significantly different (P<0.05).

Plate 1: Liver of fish fed on inulin 2.5g with the two sorghum levels, where (a-15% sorghum), showing mild focal hepatic vacuolar degeneration (vd) and (b-30% sorghum) showing mild congestion (c) of blood vessels and vacuolation of hepatocyte (H&E,×40).

Plate 2: Liver of fish fed on inulin 5g with the two sorghum levels, where (a-15% sorghum) showing moderate focal to diffuse degeneration (vd) of hepatocytes and (b-30% sorghum) showing severely congested (c) blood vessels and diffuse vacuolar degeneration of hepatic cells (H&E,×40).

Plate 3: Liver of fish fed on inulin 7.5g with the two sorghum levels, where (a-15% sorghum) showing multi focal to diffuse degeneration (d) of hepatocytes and congestion (c) of blood vessels and (b-30% sorghum) showing severe degeneration of hepatocytes, focal hemorhage (h) and edima (e), (H&E,×40).
Plate 4: Spleen of fish fed inulin 2.5g with the two sorghum levels, where (a- 15% sorghum) showing normal structure of both white (wb) and red pulp (rb) with active prominent macrophage pulp (m) and (b-30% sorghum) showing fairly normal structure of both white and red pulp (H&E,×40).

Plate 5: Spleen of fish fed on inulin 5g with the two sorghum levels, where (a-15% sorghum) showing normal splenic structure white (wb) and red pulp (rb) and (b-30% sorghum) showing mild depletion of lymphocytes (L) with mild atrophied melanomacrophage centers (m) and white pulp (w) (H&E,×40).

Plate 6: Spleen of fish fed on inulin 7.5g with the two sorghum levels, here (a-15% sorghum) showing moderate congestion (c) of blood vessels of red pulp and (b-30% sorghum) showing depletion and necrotic (n)changes of white pulp and severe congestion of blood vessels and sinusoids(H&E,×40).
DISCUSSION

Prebiotics include indigestible fiber, which fermented by gut enzymes and commensal bacteria and its beneficial effects are due to the by-products produced from fermentation. Inulin's are a group of naturally occurring polysaccharides that can be used in fish feeding. It's also contain fiber, which cannot be digested by the digestive enzymes in the gastro-intestinal tract, but has an unrivaled action in the gut, increasing the intestinal flora such as lactic acid bacteria and it has great benefit on immune-modulating effects[39].

In the present study, using inulin prebiotic showed enhancing in sorghum utilization of Nile tilapia diets. The less doses of inulin with low level of sorghum demonstrated the best indices parameters comparing to high doses.

The enhancement in growth and nutrient utilization parameters especially in fish fed at (2.5g/kg inulin+15% sorghum) diet were comparable with the previous results on tilapia Oreochromis niloticus[40], turbot Psetta maxima[41] and trout Oncorhynchus mykiss[42]. In contrast with the previous findings, other studies didn't show any effect of inulin on growth performance of some species as: hybrid tilapia, Atlantic salmon (Salmo salar), belgure Huso huso and carp[43,44,45,46]. The contradictory results reported of inulin supplementation in fish feeding probably due to different dosage levels of inulin, it's fermentability by the gut microbiota and different morphology of intestinal[47].

In the present study the haematological parameters of Nile tilapia are within the normal range in this species as indicated by previous researches[48,49,50]. However, the significance increased in WBC in this study was agree with the used of inulin in the same species[48,51] and different species include Asian sea bass,[32] and, belgure Huso huso L.[33,54].

Lysozyme activity increase defense molecule of the innate immune system, which plays an important role in interpose protection against microbial intrusion[43]. In the present study lysozyme activity revealed an important effect on the immune defense of Tilapia. The elevated significant values of lysozyme activity observed in fish fed various inulin doses especially in 15% sorghum groups compared with 30% sorghum, indicates excess ability of inulin to kill pathogenic bacteria by collapse the cell wall. Comparable observation was revealed by[34,54] in Nile tilapia fed on different prebiotics. Our results are similar with the results reported in carp[40]. In contrast with these results, other researchers revealed that inulin didn’t have a good immune-stimulant effect for gilthead sea bream (Sparus aurata L.)[40]. The different reports could be referred to duration of inulin use, age, and type of treated fish.

Antioxidant enzymes comprise ACP and SOD, which constitute the first line of enzymatic defence mechanism contra free radicals in organisms. The increased Alternative complement pathway (ACP) activity might be attributed to the enhanced liver function, which was supported by the fact that liver is the main source of sequel proteins[40]. The activity of Superoxide dismutase (SOD) is the important biochemical parameters for antioxidant defense[41]. The assay of these activities can indicate the antioxidant condition of fish and serve as biomarkers of oxidative stress in the fish[42]. SOD is a cytosolic enzyme that is specified at sweeping super oxide radicals and is embroiled in defensive mechanisms in tissue injury, subsequent with oxidative process and phagocytosis[43].

Similarly, a significance increase in ACP activity was reported in gilthead sea bream fed inulin supplemented diet[49]. Higher Significant SOD activity was obtained at 10 g/kg prebiotic fructo-oligosaccharide addition to Asian seabass juveniles diet after feeding for 45 days[52]. On the contrary, the values of ACP, SOD and lysozyme activities were didn’t affect by supplemented FOS in Triangular bream (Megalobrama terminalis) diet[44].

To date, no study has been conducted at the effect of inulin on resistance of parasitic infection in fish, but some reports pointed to that some herbs and prebiotic are high sources of immune-enhancing materials, which not only stimulate the obtained immune response by rising the diseases resistance, but also increase innate, humoral and cellular defense mechanisms[45,46,47,48]. This finding suggests that probiotic plays a positive role in improving fish health and immune response against disease infection as has been recorded by[49,50,51]. In addition the carvacrol or thymol in herbal plant reinforce various health functions and increased disease resistance in fish[49,52,53], Moreover, the beneficial effects of garlic immersion treatments on parasitic infection were illustrated in some research, where the research by[54] observed a positive effect with bathing European eel (Anguilla anguilla) at 200μL/L squeezed garlic for 24 hr in treating trichodiniad. In the same vein, the immersion of each garlic aqueous extract and garlic oil were reduced infection rates by trichodinids and gyrodactylids in juvenile tilapia (Oreochromis niloticus)[55]. Also the use of garlic extract as 50 or 150 g/ kg feed in barramundi diet has preventative effect against Neobenedenia sp[56]. The use of 20 % garlic in diet guppies (Poecilia reticulata) was found to reduced significantly infection in guppies fish[57]. Therefore, in accordance with the stated facts, the resistance of tilapia in present experimental and less infection by ectoparasite probably as a result of immunity increased in fish fed with inulin.

Histological examinations divulge that inulin supplementation shown an enhancement in liver and spleen structure especially at low doses of inulin in the two inclusion levels of sorghum. These observation reflect the beneficial effect of inulin in these organs. The same finding was reported in liver of Asian sea bass[58]. Similar to the present results other works recorded that addition of each inulin at (5.0g/kg) and Jerusalem artichoke (5.0 or 10.0 g/kg) shown an increase in villus height intestine of juvenile Nile tilapia[39].
Body lipid composition of Nile tilapia was significantly increased in fish fed high doses of inulin (5.0 and 7.5 g inulin) accompanied by 30% sorghum diets. This finding agrees with the use of other probiotic in tilapia[84,85,86] and rainbow trout[80]. In contrast of these results other researches not recorded any effect of inulin or other prebiotic supplementation diets on body composition of some fish as trout Salmo gairdneri, sea bass, common carp, giant sturgeon and Atlantic salmon[42,44,81,82,83,84,85,86].

CONCLUSION

The present results showed that inulin supplementation at low dose (2.5g/kg diet) in the two levels of sorghum inclusion (15 & 30%) in dietary Nile Tilapia, enhancing growth performance, immuno-haematological indices, parasitic infection and each of liver and spleen structure of Nile tilapia fingerlings. On the other hand, further studies are required to conclusively ascertain the effect of inulin supplementation for increase the immune responses and disease resistance in fish.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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تأثير التغذية بالانولين بريبيوتك على معدل أداء النمو، مؤشرات مناعة الدم والعدوى الطفيلية لإصبعيات البلطى النيلي

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مع انتشار المزارع السمكية بصورة كبيرة وبزيادة استخدام المضادات الحيوية التي تؤثر على البيئة وتقضى على البكتيريا النافعة بأسماك النيل، كان الاتجاه إلى استخدام بعض المركبات الطبيعية مثل البريبوتك ومنها الانولين.

هدف البحث: هدف الدراسة تقييم النسب المستخدمة من الانولين كبريبوتك طبيعي على معدل النمو، الكفاءة الغذائية، تركيب الكبد للعقارية، التأثير المناعي والإصابة الطفيلية لإصبعيات البلطى النيلي.

مواد وطرق البحث: وُزعت الأسماك في 18 حوض زجاجي بسعة (75×40×35cm) باستخدام 6 معاملات تجريبية. وُضعت كل معاملة بثلاث مكرات وسُكنت الأسماك بمعدل 25 سمًا/حوض ووزن أولى (24±9.3 جم). طُبّقت 6 علاقات باستخدام 3 مستويات من الانولين وهي (0.5، 1.0، 1.5جم/كل كجم) مع مستويين من الذرة الرفيعة (135±42جم) و(276±75جم) وذلك في مرحلة النمو. تم إجراء الرياض لدراسة اثر العلاج المستخدمة على معدلات الاداء، قياسات الدم، التركيب الكيميائي لجسم الأسماك ولطقاطس هستولوجية لكل من الكبد والطحال لأسماك البلطى النيلي. وتم تحليل الدم من كرات الدم الحمراء، البيضاء، الهيموجلوبين والهيماتوكريت وتم قياس مؤشرات المناعة المختلفة مثل نشاط إنزيمات الليزوزيم، SOD وACP والدورة المناعية بجميع معاملات الإصابة الطفيلية.

توصيات: أوضحت نتائج الدراسة أن أعلى معدل أداء للنمو وكفاءة غذائية حدثت مع المستويات المنخفضة من الانولين. Alternative Complement Pathway (ACP) و Superoxide Dismutase Activity (SOD) و Lysozyme activity

النتائج: وُجِدَت نتائج الدراسة أن أعلى معدل أداء للنمو وكفاءة غذائية حدثت مع المستويات المنخفضة من الانولين. وتحديد النسبة (0.5 جم/كجم) مع مستويات من الانولين (1.0، 1.5 جم/كل كجم) بزيادة نسبة تأثير المعاوقية عند مستوى انولين والفيلية. وكشفت النتائج أية انخفاض نشاط إنزيمي (ACP) و Simultaneous Analysis (SOD)

من الانولين واحتلف معدل نشاط إنزيم Lysozyme activity بين مستويين الذرة الرفيعة حيث ارتفعت نسبة عند مستوي انولين 10% مع الثلاث مستويات من الانولين وانخفاض نسبة مع مستوى متوسط 30% ذرة رفيعة في حين لم يتأثر تركيب جسم الأسماك معنويًا من المادة النافعة للبروتين والدهون وتم رصد عدوى متصورة في المستويات عالية من الانولين، كما أشارت نتائج الفحص الهستولوجي إلى تحسن تركيب الكبد والطحال للأسماك مع مستويات الانولين المنخفض علاوة على تحسن مقاومة للعدوى الطفيلية للأسماك مع كل مستويات.
بالاعتماد على معدلات النمو، الكفاءة الغذائية، التركيب الهيستولوجي للكبد والطحال، قياسات مناعة الدم، تركيب الجسم والإصابة الطفيلية أشارت النتائج إلى تحسن في القياسات السابقة عند المستويات المنخفضة من الإنولين 2.5 جم مع 5% ذرة رفيعة، كما أشارت نتائج الفحص الهيستولوجي إلى تحسن تركيب الكبد والطحال للأسماك مع مستوى الإنولين المنخفض علاوة على تحسن ومقاومة للعدوى الطفيلية للأسماك مع كل مستوى الإنولين وعليه فإنه يمكن استخدام مستوى منخفض من الإنولين (0.5 جم/كم مل) لإصبعيات البلطي النيلية.

الخلاصة: بالأعتماد على معدلات النمو، الكفاءة الغذائية، التركيب الهيستولوجي للكبد والطحال، قياسات مناعة الدم،