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

Original Article

Abdel Moneim Abdel Sadek Mahdy Yones[1], Ismail Abdel-Monam Mohamed Eissa[2], Mahi Abd El- Fattah Ali Ghobashy[3] and Samar Samir Marzok[1]

[1]*National Institute of Oceanography and Fisheries, Egypt*
[2]*Faculty of Veterinary Medicine -Suez Canal University, Egypt*
[3]*Faculty of Science-Suez Canal University, Egypt*

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 ecotoparasitic 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 ecoparasitic 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 dietary Nile Tilapia, enhancing growth performance, immuno-haematological indices, parasitic infection and each of liver and spleen structure of Nile tilapia fingerlings.

Received: 31 July 2019, **Accepted:** 04 August 2019

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

Corresponding Author: Abdel-Moniem Abdel Sadek Yones, PhD, National Institute of Oceanography and Fisheries, Egypt, **Tel.**: +20 1003473960, **E-mail:** yones_55200010@yahoo.com

ISSN: 1110-0559, Vol. 43, No. 1

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[8,9,10]. In addition, anti-biotic drugs can also destroyed beneficial bacteria[11] and increase the indulgence of pathogenic bacteria[12]. Many researchers concluded that probiotics were useful in the growth performance and immune function of aquatic animals[13,14].

From a long time, antibiotic agents were commonly applied for restrain bacterial diseases in commercial aquaculture projects[15]. In 2006, the European Union

 DOI: 10.21608/ejh.2019.15495.1152

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 and10.0g/kg diet. The diets were given to sex groups of fish as: group1 contain inulin 2.5g and sorghum 15% (In 2.5g + S15%), group 2, inulin 5.0g and sorghum 15% (In5.0g + S15%), group 3, inulin7.5g and sorghum 15% (In 7.5g + S15%), group 4, inulin2.5g 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.

Table 1: Feed formulation and chemical analysis of experimental diets

Diets[1]	Sorghum and Inulin levels %					
	S15	S15	S15	S30	S30	S30
Fish meal	4	4	4	6	6	6
Poultry-by product meal	8	8	8	10	10	10
Soybean meal	10	10	10	20	20	20
Sunflower seed meal	50	50	50	26	26	26
Sorghum meal	15	15	15	30	30	30
Fish oil	3	3	3	3	3	3
Cotton seed oil	3	3	3	3	3	3
Microcrystalline cellulose	5	5	5	-	-	-
Inulin	0.25	0.50	0.75	0.25	0.50	0.75
Vitamin min. mix[2]	1.75	1.50	1.25	1.75	1.50	1.75
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)[3]	18.84	18.78	18.84	18.78	18.84	18.78
Metabolizable (ME/kg diet)[4]	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; cholecolciferol 0.1; tocopheryl acetate 34.2; menodione 22; thiamin 5.6; riboflavin 12; pyridoxine 4.5; calcioum panthothenate 14.1; p-aminobenzoic acid 40; cyanocobalamin 0.03; niacin 30; biotin 0.1; choline chloride 350; folic acid 1.5; inositol 50;canthaxanthin 10;butylated hydroxytoluene 1.5; butylated hydroxyanisol 1.5.; $CaHPO_4$,$2H_2O$ 29.5; Ca (H_2PO_4)2 H_2O 217; $NaHCO_3$ 94.5; $Na_2SeO35H_2O$ 0.011; Kci 100; Nacl 172.4; Ki 0.2; Mgcl2 63.7; MgSO4 34.3; MnSO4 2; $FeSO4H_2O$ 10; $CuSO4\ 5H_2O$ 0.4; ZnSO4 10.

[3]Gross energy (MJ kg-1 diet) was calculated by using the following calorific values: 23.9, 39.8 and 17.6 kjg-1 diet for protein, either extract and nitrogen free extract, respectively[25].

[4]Metabolizable energy (MJ kg-1 diet) was calculated by using the following calorific values: 18.9, 35.7 and 14.7 kj g-1 diet for protein, either extract and nitrogen free extract, respectively[25].

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[26]. 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[27]. Contents of tannins in sorghum meal were determined using a modified version method[28].

Haematological Parameters

Red blood cell count, White blood cell count, haematocrit and haemoglobin content were detected through the method described by[29].

Immunological Indices

The lysozyme activity level was determined by the turbidimetric assay[30]. Alternative complement pathway (ACP) was performed as described by[31]. Superoxide dismutase activity (SOD) was determined as described by[32].

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[33], 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 ecoparastic trial was done after finishing the growth trial, where 20 fish from each treatment were exposed to ectoparastic 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 fish, then transmission of parasites by cohabitation (CT). The initial parasite burden of donor (D) fish will determine by examining fish before starting the cohabitation, and receptor fish (R) will mark with passive integrated transponder (PIT) tags to differentiate them. PIT-tags will inject into the dorso-lateral musculature of the Fish.

External examination of different sites of fish as eyes, opercula (gill cover, gills, fins and skin surface were done through dissecting microscope to detect any species of parasitic or worms in the above organs and the identification of the parasites was undertaken according to[34].

Clinical Examinations

The fish behaviour, movements of the operculum, feeding and any clinical abnormalities like abdominal distension, skin pigmentation, emaciation, skin lesions, wounds, petechial hemorrhages were examined according to[35].

Statistical Analysis

The current data of the present study were analyzed using two-ways Analysis of Variance (ANOVA) system for testing significance among groups[36]. The comparisons between values were done by Duncan's Multiple Range test according to[37]. All analysis were performed using SPSS version 20,(2016) SPSS Institute, Cary, NC, USA[38]. Results are considered significantly at significant levels of 0.05, ($P \leq 0.05$).

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 vacuolar 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 liver showed in (a-15% sorghum) a moderate focal to diffuse degeneration of hepatocytes and (b-30% sorghum) showed severely congested blood vessels and diffuse vacuolar degeneration of hepatic cells (plate 8). 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 hepatoctes, 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 vacuolar 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 liver showed in (a-15% sorghum) a moderate focal to diffuse degeneration of hepatocytes and (b-30% sorghum) showed severely congested blood vessels and diffuse vacuolar 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 hepatoctes, 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 prominant 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)

Parameters	Treatments					
	S15+2.5 g	S15+5.0g	S15+7.5g	S30+2.5g	S30+5.0g	S30+7.5g
Initial weight (g/fish)	9.31^{a}±0.25	9.14^{a}±0.25	9.22^{a}±0.25	9.63^{a}±0.25	9.42^{a}±0.25	8.83^{a}±0.25
Final weight (g/fish)	63.63^{a}±2.25	59.92^{b}±2.54	60.96^{b}±2.22	50.54^{c}±2.33	48.44^{c}±2.16	45.28^{d}±2.18
Total weight gain (g/fish)	54.30^{a}±0.32	50.80^{b}±0.22	51.76^{b}±0.26	40.94^{c}±0.18	39.04^{c}±0.25	36.48^{d}±0.28
Specific growth rate	2.13^{a}± 0.04	2.10^{a}±0.03	2.11^{a}± 0.05	1.84^{b}± 0.04	1.82^{b}±0.03	1.72^{b}±0.02
Condition factor (g/cm3)	1.86^{a}±0.17	1.77^{a}±0.12	1.79^{a}±0.14	1.50^{b}±0.12	1.48^{b}±0.14	1.44^{b} ±0.12
Survival rate (%)	99	98	98	98	98	97
Feed consumed(g)	83.0	85.0	91.0	93.0	90.0	88.0
Feed conversion ratio	1.53^{a}±0.35	1.67^{b}±0.33	1.76^{b}±0.26	2.27^{c}±0.23	2.31^{c}±0.34	2.41^{c}±0.23
Protein efficiency ratio	2.15^{a}±0.23	1.99^{b}±0.21	1.87^{b}±0.34	1.46^{c}±0.32	1.43^{c}±0.26	1.34^{c}±0.25
Net protein Utilization (%)	33.48^{a}±0.22	30.41^{b}±0.21	29.55^{b}±0.28	21.84^{c}±0.26	21.37^{c}±0.25	20.01^{c}±0.21
Hepatosomatic index (HSI %)	1.8^{a}±0.11	1.82^{a}±0.12	1.74^{a}±0.14	1.68^{a}±0.12	1.78^{a}±0.14	1.76^{a}±0.11

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)

Parameters	Treatments					
	S15+2.5 g	S15+5.0g	S15+7.5g	S30+2.5g	S30+5.0g	S30+7.5g
Red blood cell, RBC×10^{-9}/L	1.91 ±0.1	1.87 ±0.2	1.92 ±0.1	1.89 ±0.2	1.88 ±0.1	1.92 ±0.2
White blood cell, WBC×10^{-12}/L	96.2a±1.2	94.5a±1.5	95.4a±1.4	83.0b±1.3	82.2b±1.6	81.5b±1.2
Haematocrit, Hct/%	32,0±0.8	31.5 ±0.6	30.8 ±0.8	31.0 ±0.6	31.2 ±0.5	31.4 ±0.8
Haemoglobin, Hb g^{-1}/ L	7.2 ±0.2	7.1 ±0.4	7.4 ±0.2	7.2 ±0.4	7.2 ±0.2	7.1 ±0.1

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)

Parameters	Treatments					
	S15+2.5 g	S15+5.0g	S15+7.5g	S30+2.5g	S30+5.0g	S30+7.5g
Lysozyme activity (mg/dl)	1.99^{a} ±0.2	1.95^{a} ±0.26	1.94^{a}±0.32	1.56^{b}±0.26	1.54^{b}±0.34	1.65^{b}±0.25
Alternative complement pathway (ACP) (Um/L)	65.0^{c} ±1.4	75.0^{b} ±1.2	80.0^{a} ±1.6	66.0^{c}±1.5	77.0^{b}±1.2	81.0^{a}±1.2
Superoxide dismutase (SOD) (Um/L)	50.0^{c} ±1.2	55.0^{b} ±1.1	60.0^{a} ±1.2	51.0^{c}±1.2	56.0^{b}±1.4	61.0^{a}±1.5

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

Treatments	No of treated fish (R) non infected	No of donor fish (D) infected	No of infested fish from R and D	Morbidity %
Control	10	10	9	45
S15+2.5	10	10	3	15
S15+5.0	10	10	3	15
S15+7.5	10	10	3	15
S30+2.5	10	10	2	10
S30+5.0	10	10	3	15
S30+7.5	10	10	3	15

Table 6: Proximate chemical composition of Nile tilapia after feeding on different inulin and sorghum diets (Mean±SD n=3)

Parameters	Treatments					
	S15+2.5 g	S15+5.0g	S15+7.5g	S30+2.5g	S30+5.0g	S30+7.5g
Dry matter	26.92±0.42	26.85±0.38	27.53±0.39	26.51±0.28	28.35±0.34	28.58±1.15
Crude protein	15.62±0.21	15.42±0.54	15.81±0.21	15.17±0.22	15.15±0.48	15.13±0.81
Crude lipid	6.15b±0.83	6.21b± 0.30	6.61b± 0.08	6.34b± 0.22	8.16a± 0.11	8.34a±0.26
Ash	5.15±0.23	5.22± 0.25	5.11± 0.16	5.00± 0.21	5.04± 0.19	5.11± 0.28

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

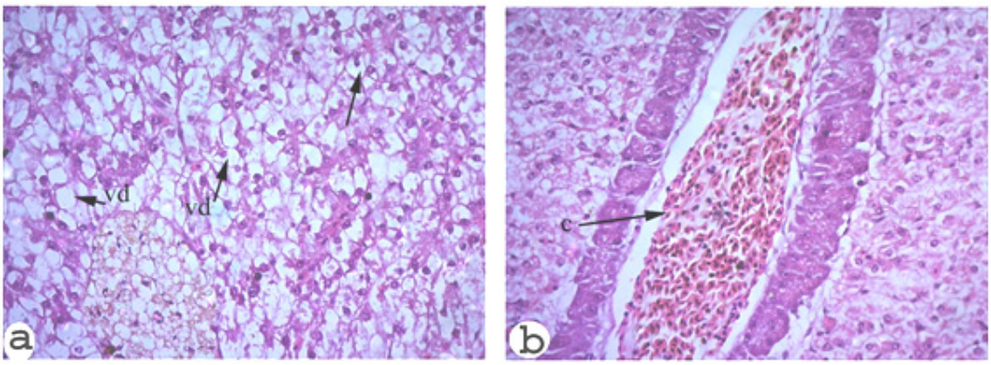

Plate 1: Liver of fish fed on inulin 2.5g with the two sorghum levels, where (a-15% sorghum), showing mild focal hepatic vacuolar degeneration (vd) and (b-30% sorghum) showing mild congestion (c) of blood vessels and vacuolation of hepatocyte (H&E,× 40).

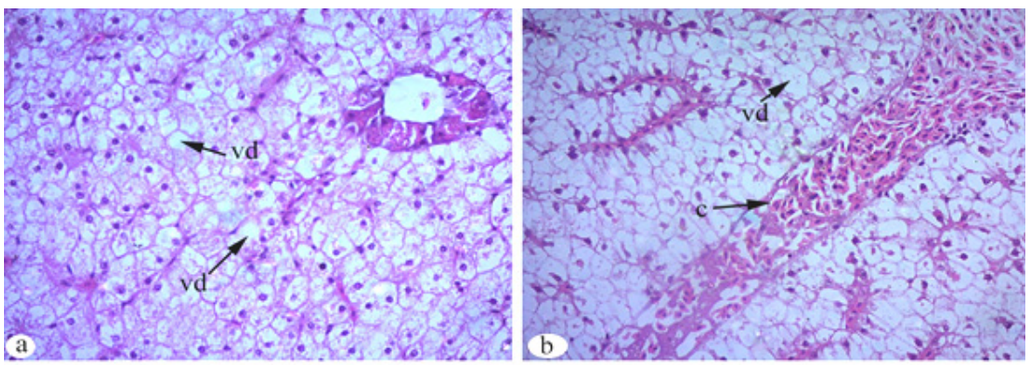

Plate 2: Liver of fish fed on inulin 5g with the two sorghum levels, where (a-15% sorghum) showing moderate focal to diffuse degeneration (vd) of hepatocytes and (b-30% sorghum) showing severely congested (c) blood vessels and diffuse vacuolar degeneration of hepatic cells (H&E,×40).

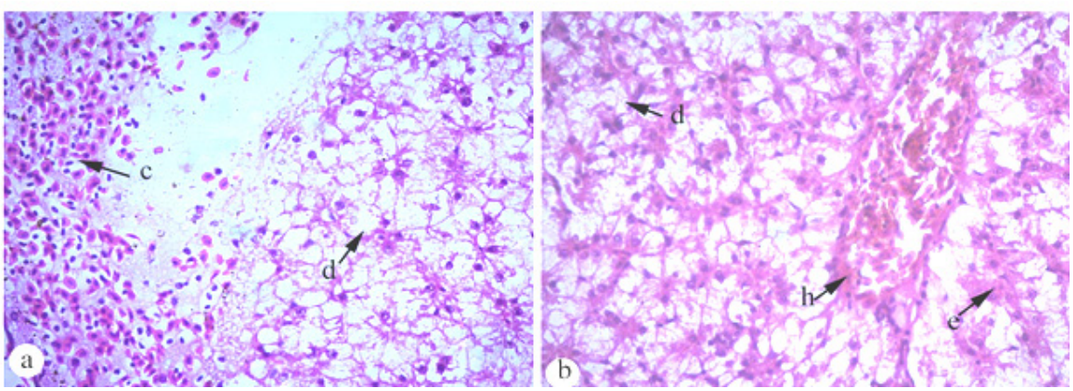

Plate 3: Liver of fish fed on inulin 7.5g with the two sorghum levels, where (a-15% sorghum) showing multi focal to diffuse degeneration (d) of hepatocytes and congestion (c) of blood vessels and (b-30% sorghum) showing severe degeneration of hepatocytes, focal hemorhage (h) and edima (e), (H&E,×40).

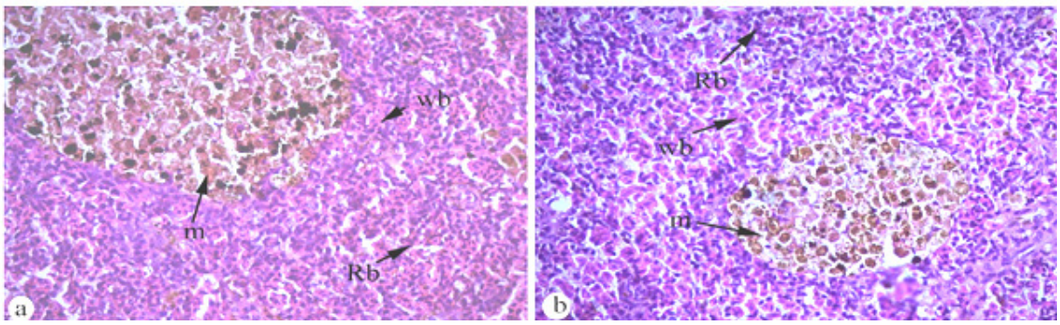

Plate 4: Spleen of fish fed inulin 2.5g with the two sorghum levels, where (a- 15% sorghum) showing normal structure of both white (wb) and red pulp (rb) with active prominent macrophage pulp (m) and (b-30% sorghum) showing fairly normal structure of both white and red pulp (H&E,×40).

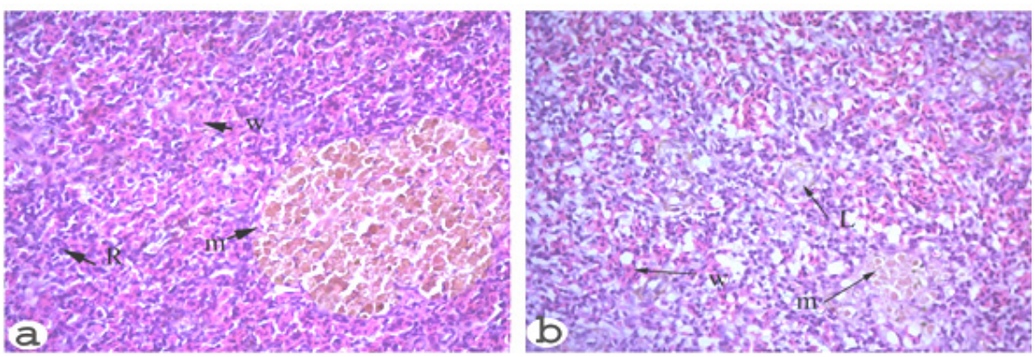

Plate 5: Spleen of fish fed on inulin 5g with the two sorghum levels, where (a-15% sorghum) showing normal splenic structure white (wb) and red pulp (rb) and (b-30% sorghum) showing mild depletion of lymphocytes (L) with mild atrophied melanomacrophage centers (m) and white pulp (w) (H&E,× 40).

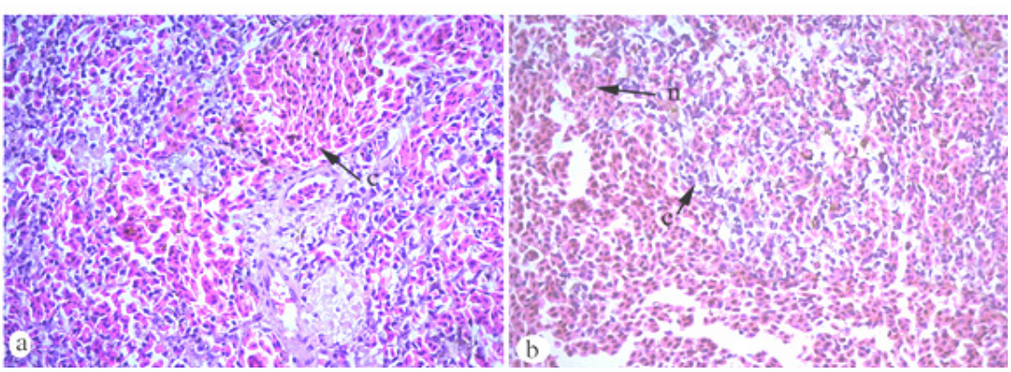

Plate 6: Spleen of fish fed on inulin 7.5g with the two sorghum levels, here (a-15% sorghum) showing moderate congestion (c) of blood vessels of red pulp and (b-30% sorghum) showing depletion and necrotic (n)changes of white pulp and severe congestion of blood vessels and sinusoids(H&E,×40).

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 unrivalled 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 current 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), belguge 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[40,51] and different species include Asian sea bass,[52] and, belgu Huso huso L.[53,54].

Lysozyme activity increase defense molecule of the innate immune system, which plays an important role in interpose protection against microbial intrusion[55]. 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[56,57] in Nile tilapia fed on different probiotics. Our results are similar with the results reported in carp[58]. 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.)[59]. 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[60]. The activity of Superoxide dismutase (SOD) is the important biochemical parameters for antioxidant defense[61]. The assay of these activities can indicate the antioxidant condition of fish and serve as biomarkers of oxidative stress in the fish[62]. 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[63].

Similarly, a significance increase in ACP activity was reported in gilthead sea bream fed inulin supplemented diet[59]. 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[64].

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[55,65,66,67]. This finding suggests that probiotic plays a positive role in improving fish health and immune response against disease infection as has been recorded by[68,69,70,71]. In addition the carvacrol or thymol in herbal plant reinforce various health functions and increased disease resistance in fish[72,73,74]. Moreover, the beneficial effects of garlic immersion treatments on parasitic infection were illustrated in some research, where the research by[75], observed a positive effect with bathing European eel (Anguilla anguilla) at 200µl/L squeezed garlic for 24 hr in treating trichodiniasis. 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)[76]. Also the use of garlic extract as 50 or 150 g/kg feed in barramundi diet has preventative effect against Neobenedenia sp[77]. The use of 20 % garlic in diet guppies (Poecilia reticulata) was found to reduced significantly infection in guppies fish[78]. Therefore, in accordance with the stated facts, the resistance of tilapia in present experimental and less infection by ectoparsite 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[52]. 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[79].

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[50] 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.

REFERENCES

1. Food and Agriculture Organization (FAO). (2014). The State of World Fisheries and Aquaculture 2014. Foodand Agriculture Organization of the United Nations, Rome, Italy.
2. Welker, T.L. and Lim, C. (2011). Use of probiotics in diets of tilapia. Journal of Aquaculture Research & Development,S1-014,8.
3. Polakof, S., Panserat, S., Soengas, J.L. and Moon, T.W., (2012). Glucose metabolism in fish: a review. J. Comp. Physiol. 182B, 1015-1045.
4. Ezzat, E. M.; Ali, M.A. and Mahmoud (2010). Agronomic performance, genotype× environment interaction and stability analysis of grain sorghum (Sorghum bicolor L. Moench). Asian J. Crop Sci.,2(4)250-260, ISSN 1994-7879.
5. Mundi index (2018). Sorghum production in Egypt. www.worldagriculturalproduction.com/Crops/sorghum.aspx.
6. Miller magazine (2019). Global sorghum and millet. Issue, 115, info@millermagazine.com.
7. Abd-El-Azem (2016). Effect of partial or complete replacement of yellow corn (Zea mays) by sorghum (Sorghum bicolor) and Lacto cel- con as probiotic on growth performance, feed utilization and body composition in mono sex Nile tilapia (Oreochromis niloticus). Msc. Thesis Faculty of agriculture, Al-Azhar Univ. Cairo.
8. Watts, M., Munday, B.L. and Burke, C.M. (2001). Immune responses of teleost fish. Aust. Vet.J.79:570–574.
9. Cheng, A.C., Cheng, S.A., Chen, Y.Y., Chen, J.C. (2009). Effects of temperature change on the innate cellular and humoral immune responses of orange-spotted grouper Epinephelus coioides and its susceptibility to Vibrio alginolyticus. Fish Shellfish Immunol., 26:768–772.
10. Cha J-H., Rahimnejad S., Yang S-Y., Kim K-W. and Lee KJ. (2013). Evaluation of Bacillus spp. as Dietary additives on growth performance, innate immunity and disease resistance of Olive Flounder (Paralichtys olivaceus) against Streptococcus iniae as water additive. Aquaculture 402-403, 50–57.
11. Sahu, M., Swarna kumar, N., Siva kumar,K.,Thangaradjou,T. and Kannan,L.(2008). Probiotics in aquaculture: importance and future perspectives. Indian J. Microbiol, 48: 299–308.
12. Balcazar, J.L., De Blas, I., Ruiz-Zarzuela, I., Cunningham, D., Vendrell, D. and Muzquiz, J.L.(2006).The role of probiotics in aquaculture. Vet. Microbiol. 114:173–186.
13. Talpur, A.D., Munir, M.B., Mary, A. and Hashim, R.(2014). Dietary probiotics and prebiotics improved food acceptability, growth performance, haematology and immunological parameters and disease resistance against Aeromonas hydrophila in snakehead (Channa striata) fingerlings. Aquaculture, 426-427:14-20.
14. Wang,J.L.,Meng,X.L.,Lu,R.H.,Wu,C.,Luo,Y.T.,Yan,X..,Li,X.J., Kong, X.H. and Nie, G.X. (2015). Effects of Rehmannia glutinosa on growth performance, immunological parameters and disease resistance to Aeromonas hydrophila in common carp (Cyprinus carpio L.). Aquaculture, 435:293–300.
15. Faggio, C., Fazio, F., Marafioti, S., Arfuso, F. and Piccione, G. (2015). Oral administration of Gum Arabic: Effects on hematological parameters and oxidative stress markers in Mugil cephalus. Iranian Journal of Fishery Science, 60:60–72.
16. Maron,D.,Smith,T. and Nachman, K.(2013). Restrictions on antimicrobial use in food animal production: an international regulatory and economic survey. Global Health 9, 48.
17. Cabello, F.C., Godfrey, H.P., Tomova, A., Ivanova, L., Dolz, H., Millanao, A. and Buschmann, A.H. (2013). Antimicrobial use in aquaculture re-examined: its relevance to antimicrobial resistance and to animal and human health. Environmental Microbiology, 15:1917–1942.
18. Gatlin, D.M. (2015). Prebiotics Dietary nutrients, additives, and fish health (pp.271-281). New York, NY: John Wiley & Sons Inc.
19. Carbone, D. and Faggio, C. (2016). Importance of prebiotics in aquaculture as immune stimulants. Effects

on immune system of Sparus aurata and Dicentrarchus labrax. Fish & Shell fish Immunology,54:172–178.

20. Ringo, E., Olsen, R. E., Gifstad, T.O., Dalmo, R. A., Amlund, H., Hemre, G.I. and Bakke, A.M. (2010). Prebiotics in aquaculture: a review. Aquacult. Nutr., 16:117–136.
21. Van Loo, J., Cummings,J.,.Delzenne, N.,. Franck, A., Hopkins, M., MacFarlane, G., Newton, D., White,L.A., Newman, M.C., Cromwelland, G.L., and Lindemann, M.D. (2002).Brewers dried yeast as a source of mannan oligosaccharides for weaning pigs. Journal of Animal Science, 80:2619–2628.
22. Blanca, Partida-Arangure, O., Luna-Gonzalez,A.,Jesus,Fierro-Coronado,A.,Del Carmen, Flores-Miranda, M.A. and Hector, Gonzalez-Ocampo, A. (2013). Effect of inulin and probiotic bacteria on growth, survival, immune response, and prevalence of white spot syndrome virus (WSSV) in Litopenaeus vannamei cultured under laboratory conditions. African Journal of Biotechnology, 12:3366–3375.
23. Burr, G., Hume, M., Ricke, S., Nisbet, D. and Gatlin, D.M. (2010). In vitro and in vivo evaluation of the prebiotics GroBiotic-A ,inulin, mannan oligosaccharide and galacto oligosaccharide on the digestive microbiota and performance of hybrid striped bass (Morone chrysops×Morone saxatilis). Microbial Ecology,59:187–198.
24. Hrubec, T.C., Cardinale, J.L. and Smith, S.T. (2000). Hematology and Plasma Chemistry Reference Intervals for Cultured Tilapia (Oreochromis Hybrid). Veterinary Clinical Pathology, 29 (1) :1-12.
25. National Research Council, NRC. (2011). Nutrient Requirements of fish and shrimp. National Academy Press, Washington, D.C., USA.
26. AOAC(2006). Official Methods of Analysis of AOAC International, 18th ed. Rev.1. AOAC International. (Gaithersburg, MD, USA.).
27. Folch, J., Lees, M. and Sloane Stanley, G.H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 226:497-509.
28. Price, M. S., Van Scoyoc, S., and Butler, L. G., (1978) A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. J. Agr. Food Chem. 26: 1214- 1218.
29. Qiang,J.,Yang, H., Wang, H. ,Kpundeh, M.D. and Xu, P.(2012).Interacting effects of water temperature and dietary protein level on hematological parameters in Nile tilapia juvenile, Oreochromis niloticus (L.) and mortality under Streptococcus iniae infection. Fish & Shellfish Immunology 34, 8–16.
30. Ellis, AE (1990). Serum anti-proteases in fish and lysozyme assays. In: Stolen JS, Fletcher TC, Anderson DP, Roberson BS, Van Muiswinkel WB (eds) Techniques in fish immunology. SOS Publications, Fair Haven, pp:95-103.
31. Sunyer, J. O., and Tort, L. (1995). Natural hemolytic and bactericidal activities of sea bream Sparus aurata is affected by the alternative complement pathway. Veterinary Immunology. Immuno pathology,45:333–345.
32. Misra, H.P., and Fridovich, I. (1972). The role of superoxide anion in the autooxidation of epinephrine and a simple assay of superoxide dismutase. Journal of Biological Chemistry, 247:3170–3175.
33. Bancroft, J. D. and Cook, H.C. (1996). Manual of histological techniques and their diagnostic application. Churchill Livingstone, London, UK., pp: 289- 305.
34. Yardimci ,B. and Aydin, Y.(2011). Pathological findings of experimental Aeromonas hydrophila infection in Nile tilapia (Oreochromis niloticus). Ankara Univ. Vet. Fak Derg, 58:47-54.
35. Hoffman, G.L. (1999). Parasites of North America Freshwater 2nd ed. Comostock publishing Associates, Ithaca, New York.
36. Snedecor, G.W. and Cochran, W. G. (1989). Statistical Methods, Eighth Edition, Iowa State University Press.
37. Duncan, D. B. (1955): Multiple range and multiple F tests. Biometrics,11:1–42.
38. SPSS (2016). SPSS Statistics for Windows, Version, 20, Chicago:SPSS Inc, USA.
39. Vogt, L.,Meyer,D.,Pullens,G.,Faas,M.,Smelt,M.,Venema,K. and Ramasamy, U. (2015). Imunological properties of inulin-type fructans. Crit. Rev. Food Sci. Nutr.,55:414-436.
40. Ibrahem, MD, Fathi M, Mesalhy,S. and Abd El-Aty, AM. (2010). Effect of dietary supplementation of inulin and vitamin C on the growth, hematology, innate immunity, and resistance of Nile tilapia (Oreochromis niloticus). Fish Shellfish Immunol,29:241 e 6.
41. Mahious, A.S., Gatesoupe, F.J., Hervi, M., Meˆ tailler, R. and Ollevier, F. (2006). Effect of dietary inulin and oligosaccharides as prebiotics for weaning turbot, Psetta maxima (Linnaeus, C 1758). Aquacult. Int., 14:219-229.
42. Ortiz, L., Rebole´ , A., Velasco, S., Rodrı´guez, M., Trevin˜ o, J., Tejedor, J. & Alzueta, C. (2013). Effects of inulin and fructooligosaccharides on growth performance, body chemical composition and intestinal microbiota of farmed rainbow trout (Oncorhynchus mykiss). Aquacult. Nutr., 19, 475–482.

43. He, S.,Xu, G., Wu, Y., Weng, H. and Xie, H. (2003). Effects of IMO and FOS on the growth performance and non-specific immunity in hybrid tilapia. Chinese Feed, 23: 14- 15.

44. Grisdale-Helland, B., Helland, S.J. and Gatlin, D.M. (2008). The effects of dietary supplementation with mannan oligosaccharide, fructooligosaccharide orgalactooligosaccharide on the growth and feed utilization of Atlantic salmon (Salmo salar L.). Aquaculture, 283:163-167.

45. Akrami, A., Hajimoradloo, A., Matinfar, A. and Abedian Kinai, A. (2009). Effect of dietary prebiotic Inulin on growth performance, intestinal microflora, body composition and hematological performance of juvenile Beluga Huso huso (Linnaeus, 1758), Journal of the World Aquaculture Society, 40:771-779.

46. Eshaghzadeh, H., Hoseinifar. S. H., Vahabzadeh, and Ringo, E., (2015). The effects of dietary inulin on growth performance, survival and digestive enzyme activities of common carp (Cyprinus carpio) fry. Aquacult. Nut., 21:242–247.

47. Hoseinifar, S.H., Zare, P. and Merrifield, D.L. (2010). The effects of inulin on growth factors and survival of the Indian white shrimp larvae and postlarvae (Fenneropenaeus indicus). Aquacult. Res., 41:348–352.

48. Barros, M. M., Ranzani-Paiva, M. J. T., Pezzato, L. E., Falcon, D.R. and Guimaraes, I.G. (2009). Hematological response andgrowth performance of Nile tilapia fed diets contain folic acid. Aquaculture Research, 40: 895–903.

49. Weiss, D.J. and Wardrop, K.J.(2010). Schalm's Veterinary Haematology (6th edn.). Blackwell Publishing, Iowa, USA.

50. Yones, A.M, Hussein, M.S. ,Ali, A.W. and Abdel-Azem, M.A. (2019). Effect of dietary Lacto cel-con probiotic on growth performance and hematology indices of fingerlings mono-sex Nile tilapia (Oreochromis niloticus). Egyptian Journal of Aquatic Biology & Fisheries, 23(2):227 – 239.

51. Sado, R. J., Bicudo, A. J. D. A. and Cyrno, J. E. P. (2008). Feeding dietary mannan oligosaccharide to juvenile Nile tilapia (Oreochromis niloticus), has no effect on hematological parameters and showed decreased feed consumption. Journal of World Aquaculture Society, 39:821–826.

52. Ali, S.R., Kondusamy, K., Praveena, P.E.,N,kumar, S. and Musthafa, M.S. (2018). Effect of dietary prebiotic inulin on histology, immuno-haematological and biochemical parameters of Asian seabass (Lates calcarifer).Aquaculture Research, 49:2732–2740.

53. Ahmadifar, E., Akrami, R.,Ghelichi,A., and Mohammadi Zarejabad, A.(2011). Effects of different dietary prebiotic inulin levels on blood serum enzymes, haematological and biochemical parameters of great sturgeon (Huso huso) juveniles. Comparative Clinical Pathology, 20:447–451.

54. Akrami, A., Hajimoradloo, A., Matinfar, A. and Abedian Kinai, A. (2009). Effect of dietary prebiotic Inulin on growth performance, intestinal microflora, body composition and hematological performance of juvenile Beluga Huso huso (Linnaeus, 1758), Journal of the World Aquaculture Society, 40:771-779.

55. Li, W.F., Zhang, X.P., Song, W.H., Deng, B., Liang, Q. and Fu, L.Q. (2012).Effects of Bacillus preparations on immunity and antioxidant activities in grass carp (Ctenopharyngodon idellus).Fish Physiology and Biochemistry,38:1585–1592.

56. Shelby, R. A., Lim, C., Yildirim-Aksoy, M., and Delaney, M. A. (2006). Effects of probiotic diet supplements on disease resistance and immune response of young Nile tilapia, Oreochromis niloticus. Journal of Applied Aquaculture, 18:23–34.

57. Ferguson, R.M., Merrifield, D.L., Harper, G.M., Rawling, M.D. and Mustafa, S.(2010). The effect of Pediococcus acidilactici on the gut microbiota and immune status of on-growing red tilapia (Oreochramis niloticus). Journal of Applied Microbiology, 109: 851-862.

58. Mousavi, E., Mohammadiazarm, H., Seied Mohammad Mousavi, M. S., Ghatrami, R. E., (2016). Effects of Inulin, Savory and Onion Powders in Diet of Juveniles Carp Cyprinus Carpio (Linnaeus 1758) on Gut Micro Flora, Immune Response and Blood Biochemical Parameters. Turkish Journal of Fisheries and Aquatic Sciences. 16: 831-838.

59. Cerezuela, R. Cuesta, A.,Meseguer, J. and Esteban, MA. (2008). Effects of inulin on gilthead seabream (Sparus aurata) innate immune parameters. Fish Shellfish Immunol, 24:663-668.

60. Holland, M. C. and Lambris, J. D. (2002). The complement system in teleost's. Fish and Shellfish Immunology, 12:399–420.

61. Kohen, R. and Nyska, A.(2002). Oxidation of biological systems: Oxidative stress phenomena, antioxidants, redoxreactions and methods for their quantification. Toxicology Pathology, 30:620–650.

62. Hadas, E., and Stankiewicz, M.(1998).Superoxide dismutase and total antioxidant status of larvae and adults of Trichostrongylus colubriformis, Haemonchus contortus and Ostertagia circumcincta. Parasitology Research,84:646–650.

63. Olsen, R.E., Myklebust, R. ,Kryvi, H., Mayhew,T.M., and Ringo, E.(2001).Damaging effect of dietary inulin to intestinal enterocytes in Artic charr (Salvelinus alpinus L.).AquacultureResearch,32:931–934.

64. Zhang, C. N., Li, X. F., Xu, W. N., Jiang, G. Z., Lu, K. L., and Wang, L. N. (2013).Combined effects of dietary fructooligosaccharide and Bacillus licheniformis on innate immunity, antioxidant capability and disease resistance of triangular bream (Megalobrama terminalis). Fish & Shellfish Immunology,35:380–1386.

65. Galindo-Villegas, J. and Hosokawa, H. (2004). Immuno stimulants: towards temporary prevention of diseases in marine fish. Advances en Nutrition. Acuicola VII Memorias del VII Symposium International e de Nutricion Acuícola ,16-19.

66. Buentello, J.A., Neill, W.H. and Gatlin, D.M. (2010). Effects of dietary prebiotics on the growth, feed efficiency and non-specific immunity of juvenile red drum Sciaenops ocellatus fed soybean-based diets. Aquaculture Research, 41:411-418.

67. Hoseinifar, S.H., Ringo,E.,Shenavar, Masouleh, A. and Esteban, M.A. (2016). Probiotic, prebiotic and symbiotic supplements in sturgeon aquaculture: A review. Reviews in Aquaculture, 8:89–102.

68. Abdel-Tawwab, M., Abdel-Rahman, A. M. and Ismael, N.E.M. (2008). Evaluation of commercial live baker's yeast, Saccharomyces cerevisiae as a growth and immunity promoter for Fry Nile tilapia, Oreochromis niloticus (L.) challenged in situ with Aeromonas hydrophila. Aquaculture, 280:185–189.

69. Abdel-Tawwab M. (2012).The use of American Ginseng (Panaxquin quefolium) in practical diets for Nile tilapia (Oreochromis niloticus): growth performance and challenge with Aeromonas hydrophila. Journal of Applied Aquaculture 24:366–376.

70. Chi, C., Jiang, B., Yu, X.-B., Liu, T.-Q., Xia, L. and Wang, G.-X.(2014). Effects of three strains of intestinal autochthonous bacteria and their extra cellular products on the immune response and disease resistance of common carp, Cyprinus carpio. Fish Shellfish Immunol,36,9–18.

71. Irianto, A. and Austin, B. (2002). Use of probiotics to control furunculosis in rainbow trout, Oncorhynchus mykiss (Walbaum). J Fish Dis.,25:333–342.

72. Zhang,G., Gong,S., Yu,D. and Yuan, H.(2009). Propolis and Herba epimedii extracts enhance the non-specific immune response and disease resistance of Chinese sucker, Myxocyprinus asiaticus. Fish& Shell fish Immunology, 26:467–472.

73. Rattanachaikunsopon, P. and Phumkhachora, P. (2010). Assessment of synergistic efficacy of carvacrol and cymene against Edwardsiella tarda in vitro and in Tilapia (Oreochromis niloticus). African Journal of Microbiology Research, 4:420-425.

74. Volpatti. D. Chiara, B., Francesca, T. and Marco, G. (2012). Growth parameters, innate immune response and resistance to Listonella (Vibrio) anguilarum of Dicentrarchus labrax fed carvacrol supplemented diets. Aquaculture Research, 45:31-41.

75. Madsen, H. C. K., Buchmann, K., and Mellergaard, S. (2000). Treatment of trichodiniasis in eel (Anguilla anguilla) reared in recirculation systems in Denmark: Alternatives to formaldehyde. Aquaculture,186,221–231.

76. AbdEl-Galil, M.A.A., and Aboelhadid, S.M.(2012). Trials for the control of trichodinosis and gyrodactylosis in hatchery reared Oreochromis niloticus fries by using garlic. Veterinary Parasitology, 185:57–63.

77. Militz,T.A., Southgate, P. C., Carton, A.G., and Hutson, K. S. (2013). Dietary supplementation of garlic (Allium sativum) to prevent monogenean infection in aquaculture. Aquaculture, 408–409:95–99.

78. Kim,J.H., Sophie Fridman,S., Borochov-Neori,H., Sinai,T. and Zilberg, D. (2019). Evaluating the use of garlic (Allium sativum) for the remedy of Cryptocaryon irritans in guppies (Poecilia reticulata) .Aquac Res.,50:431–438.

79. Tingtam, N., Khempaka, S., Paengkoum, P. and Boonanuntanasarn, S. (2015). Effects of inulin and Jerusalem artichoke (Helianthus tuberosus) as prebiotic ingredients in diet of juvenile Nile tilapia (Oreochromis niloticus). Animal Feed Science and Tecnology,25: 120-129.

80. Yilmaz, E. Genc, M.A. and Gene, E. (2007). Effects of dietary mannanoligosaccharides on growth body composition and intestinal and liver histology of rainbow trout, Oncorhynchus mykiss. Isr. J. Aquac -Bamid., 59:182-188.

81. Wang, Y., Liu, L.Y., Tian, L. X., Zhen-Yu Du, Wang, J. T., Wang, S., and Xiao, W. P., (2005). Effects of dietary carbohydrate level on growth and body composition of juvenile tilapia, Oreochromis niloticus × O. aureus. Aquaculture Research, 2005, 36: 1408-1413.

82. Torrecillas, S., Makol, A., Caballero, M.J., Montero, D., Robaina, L., Real, F., Sweetman, J., Tort, L. and Izquierdo, M.S. (2007). Immune stimulation and improved infection resistance in European sea bass (Dicentrarchus labrax) fed mannan oligosaccharides. Fish Shellfish Immunol., 23:969-981.

83. Wu,Y.Liu, W.B., Li, H.Y., Xu, W.N. and He, J.X. (2013). Effects of dietary supplementation of fructo-oligosaccharide on growth performance, body composition, intestinal enzymes activities and histology of blunt snout bream (Megalobrama amblycephala) fingerlings. Aquaculture nutrition, 19:886-894.

84. Ebrahimi, G., Quraji, H. Khalesi, M.K., Sudagar, M., Barari, A., Zarei, D.M. and Khalil, K.H. (2012). Effects of a prebiotic, immunogen on feed utilization, body composition, immunity and resistance to (Aeromonas hydrophila) infection in the common carp (Cyprinus

caripo L.) fingerlings. J. Anim. Physiol. .Anim.Nutr., 96:591-599.

85. Mansour, M.R. Akrami, R., Ghobadi, S.H., Denji, K.A., Eza-trahimi, N. and Gharaei, A. (2012). Effect of dietary mannan oligosaccharide (MOS) on growth performance, survival, body composition and some hematological parameters in giant sturgeon juvenile (Huso huso L.). Fish Physiol. Bio-Chem., 38:829-835.

86. Roberfroid, M. and Robertson, D. (2005). Effects of inulin and oligofrucotse on health and well-being. Br J Nutr.,93: Suppl 1:S1–S168.

الملخص العربى

تأثيرالتغذية بالانولين بريبيوتك على معدل أداء النمو، مؤشرات مناعة الدم والعدوى الطفيلية لإصبعيات البلطى النيلى

عبد المنعم عبد الصادق مهدى يونس[١]، اسماعيل عبد المنعم محمد عيسى[٢]،

ماهى عبد الفتاح على غباشى[٣]، سمر سمير مرزوق[١]

[١]المعهد القومى لعلوم البحار والمصايد
[٢]كلية الطب البيطرى-جامعة قناة السويس
[٣]كلية العلوم- جامعة قناة السويس

مع انتشار المزارع السمكية بصورة كبيرة وبزيادة استخدام المضادات الحيوية التى تؤثر على البيئة وتقضى على البكتيريا النافعة بأمعاء الأسماك كان الاتجاه الى استخدام بعض المركبات الطبيعية مثل البريبوتك ومنها الانولين.

هدف البحث: هدفت الدراسة الى تقييم النسب المستخدمة من الانولين كبريبوتك طبيعى على معدل النمو، الكفاءة الغذائية، تركيب الكبد هستولوجيا، التأثير المناعى والإصابة الطفيلية لإصبعيات البلطى النيلى.

مواد وطرق البحث: وزعت الأسماك فى ١٨ حوض زجاجى سعة (35cm×40×75) باستخدام ٦ معاملات تجريبية ومُثلت كل معاملة بثلاث مكررات وسُكنت الأسماك بمعدل ٢٥ سمكة/حوض ووزن أولى (٩,٣٢ ± ٠,٢٤ جم) . كُونت ٦علائق باستخدام 3 مستويات من الانولين وهى (٥ ،٢،٥ ، ٥، ٧ جم/كجم عليقة) مع مستويين من الذرة الرفيعة ١٥ و٣٠% وغُذيت بعليقة حتى الشبع خلال ٩٠يوم من التجربة. تم اخذ العينات لدراسة اثرالعلائق المستخدمة على معدلات الاداء، قياسات الدم، التركيب الكيميائى لجسم الأسماك وتم عمل قطاعات هستولوجية لكل من الكبد والطحال لأسماك البلطى النيلى. وتم تحليل الدم من كرات الدم الحمراء، البيضاء، الهيموجلوبين والهيماتوكريت وتم قياس مؤشرات المناعة المختلفة مثل نشاط إنزيمات الليزويم، ACP و SOD وتم تعريض ٢٠ سمكة بكل معاملة الى الإصابة الطفيلية وتم عمل القياسات الاكلينيكية والفحص الميكروسكوبى للأسماك التى تعرضت للإصابة الطفيلية.

النتائج: أوضحت نتائج الدراسة أن أعلى معدل أداء للنمو وكفاءة غذائية حدثت مع المستويات المنخفضة من الانولين ٢,٥ جم/كجم عليقة كما أوضحت كرات الدم البيضاء وإنزيمات الأكسدة منAlternative Complement Pathway (ACP)) and Superoxide Dismutase Activity (SOD)) تأثراً معنويا عند مستوى (٠,٠٥) بزيادة نسبة الانولين واختلف معدل نشاط إنزيم Lysozyme activity بين مستوبين الذرة الرفيعة حيث ارتفعت نسبته عند مستوى ١٥% مع الثلاث نسب من الانولين وانخفضت نسبته مع مستوى عليقة ٣٠% ذرة رفيعة فى حين لم يتاثر تركيب جسم الأسماك معنويا من المادة الجافة, البروتين الخام والرماد بينما زاد محتوى الدهن زيادة معنوية فى المستويات العالية من الانولين، كما أشارت نتائج الفحص الهستولوجى الى تحسن تركيب الكبد والطحال للأسماك مع مستوى الانولين المنخفض علاوة على تحسناً ومقاومة للعدوى الطفيلية للأسماك مع كل مستويات.

الخلاصة: بالأعتماد على معدلات النمو، الكفاءة الغذائية، التركيب الهستولوجى للكبد والطحال، قياسات مناعة الدم، تركيب الجسم والإصابة الطفيلية أشارت النتائج الى تحسناً فى القياسات السابقة عند المستويات المنخفضة من الانولين ٢,٥ جم مع ١٥% ذرة رفيعة، كما أشارت نتائج الفحص الهستولوجى الى تحسن تركيب الكبد والطحال للأسماك مع مستوى الانولين المنخفض علاوة على تحسن ومقاومة للعدوى الطفيلية للأسماك مع كل مستويات الانولين وعليه فأنه يمكن استخدام مستوى منخفض من الانولين (٢,٥ جم/كجم عليقة) لإصبعيات البلطى النيلى.